

PROCEEDINGS  
OF THE  
NATIONAL ACADEMY OF SCIENCES, INDIA  
1963

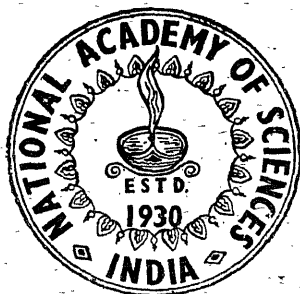
---

VOL. XXXIII

SECTION—B

PART IV

---



NATIONAL ACADEMY OF SCIENCES, INDIA  
ALLAHABAD

# THE NATIONAL ACADEMY OF SCIENCES, INDIA

(Registered under Act XXI of 1860)

Founded 1930

Council for 1963

## President

Prof. P. Maheshwari, D.Sc., F.N.I., F.B.S., F.A.Sc., F.N.A.Sc., Delhi

## Vice-Presidents

Prof. S. Ghosh, D.Sc., F.N.I., F.N.A.Sc., Jabalpur

Prof. B. N. Prasad, Ph.D., D.Sc., F.N.I., F.N.A.Sc., Allahabad

## Honorary Treasurer

Prof. R. N. Tandon, M.Sc., Ph.D., D.I.C., F.A.Sc., F.N.A.Sc., Allahabad

## Foreign Secretary

Prof. S. N. Ghosh, D.Sc., F.N.A.Sc., Allahabad

## General Secretaries

Dr. R. K. Saksena, D.Sc., F.N.I., F.N.A.Sc., Allahabad

Dr. A. G. Jhingran, M.Sc., Ph.D., F.N.I., F.G.M.S., M.M.G.I., F.N.A.Sc., Calcutta.

## Members

Prof. N. R. Dhar, D.Sc., F.R.I.C., F.N.I., F.N.A.Sc., Allahabad

Dr. A. C. Joshi, D.Sc., F.N.I., F.N.A.Sc., Chandigarh

Dr. S. Ranjan, D.Sc., F.N.I., F.A.Sc., F.N.A.Sc., Allahabad

Prof. A. C. Banerji, M.A., M.Sc., F.N.I., Allahabad

Dr. P. L. Srivastava, M.A., D.Phil., F.N.I., F.N.A.Sc., Muzaffarpur

Dr. S. H. Zaheer, M.A., D.Phil., F.N.A.Sc., New Delhi

Prof. M. D. L. Srivastava, D.Sc., F.N.A.Sc., Allahabad

Prof. Raj Nath, Ph.D., D.I.C., F.N.I., F.N.A.Sc., Varanasi

Prof. D. S. Srivastava, M.Sc., Ph.D., F.R.M.S., F.R.E.S., Sagar

The *Proceedings of the National Academy of Sciences, India*, is published in two sections : Section A (Physical Sciences) and Section B (Biological Sciences). Four parts of each section are published annually (since 1960).

The Editorial Board in its work of examining papers received for publication is assisted, in an honorary capacity, by a large number of distinguished scientists. Papers are accepted from members of the Academy *in good standing*. In case of a joint paper, one of the authors must be a member of the Academy. The Academy assumes no responsibility for the statements and opinions advanced by the authors. The papers must conform strictly to the rules for publication of papers in the *Proceedings*. A total of 50 reprints is supplied free of cost to the author or authors. The authors may have any reasonable number of additional reprints at cost price, provided they give prior intimation while returning the proof.

Communications regarding contributions for publication in the *Proceedings*, books for review, subscriptions etc. should be sent to the General Secretary, National Academy of Sciences, India, Lajpatrai Road, Allahabad-2 (India).

**Annual Subscription for both Sections : Rs. 65 ; for each Section : Rs. 35  
(including Annual Number) ; Single Copy: Rs. 7.50 ; Annual Number Rs. 5**

PROCEEDINGS  
OF THE  
NATIONAL ACADEMY OF SCIENCES  
INDIA  
1963

VOL. XXXIII

SECTION - B

PART IV

NUTRITIONAL REQUIREMENTS OF *ALTERNARIA TENUIS* AUCT.  
CAUSING LEAF SPOT DISEASE OF TOMATO

By

R. N. TANDON and C. CHATURVEDI

Botany Department, Allahabad University, Allahabad

[Received on May 14, 1962]

Nutrition of *Alternaria* species has been studied by many workers (Pawar and Patel, 1957; Tandon and Grewal, 1954; Ghosh, 1960; Thind and Gill, 1961; Tandon and Srivastava, 1958 as well as Rangaswami and Sambandam, 1961) but considerable variations in the behaviour of various isolates have been reported. Nutritional studies of *Alternaria tenuis* Auct. causing leaf spot disease of tomato have not been made so far. The present paper deals with the effect of different pH values as well as carbon, nitrogen and sulphur sources on the growth and sporulation of this organism.

**Material and Methods :**

*Alternaria tenuis* Auct. was isolated from the diseased leaves of tomato which were collected from several fields at Allahabad. Single spore cultures were prepared by the usual method. Pyrex glass wares and chemicals of highest purity were used.

On the basis of the previous experiments Asthana and Hawker's medium A\* was selected for carrying out the nutritional studies. The pH of the medium was adjusted with the help of Beckman's pH meter by adding 6 N HCl or NaOH. In order to study the effect of various carbon, nitrogen and sulphur sources, they were substituted singly by replacing the original corresponding compounds, i.e., glucose,  $\text{KNO}_3$  or  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  respectively in the basal medium. The amount of these substances was so adjusted as to furnish the same quantity of carbon, nitrogen, or sulphur which was present in the basal medium. The amount of polysaccharides (starch, dextrin and inulin) and peptone was equivalent to that of glucose and  $\text{KNO}_3$  respectively.

\*Glucose 5.0 g,  $\text{KNO}_3$  3.5 g,  $\text{KH}_2\text{PO}_4$  1.75 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.75 g, Distilled water 1000 c.c.

25 ml. medium was taken in 150 ml. Pyrex Erlenmeyer flasks and was sterilized by autoclaving for 15 minutes at 15 pounds steam pressure. After inoculations the cultures were incubated at 25°C ( $\pm 2^\circ\text{C}$ ) for 15 days. The growth records were maintained by harvesting and weighing the fungal mat after drying to a constant weight. The degree of sporulation has been indicated on the basis of the number of spores present under low power field of the microscope, e.g., few spores (1-3), poor (4-10), fair (11-20), good (21-30) and excellent (above 30). For grading the dry weights of different experiments into good, moderate and poor, the general mean of the experiment  $\pm$  critical difference (C.D.) at 5% level has been reported as moderate. The dry weights higher than the moderate have been designated as good and lower ones as poor.

#### Experimental :

1. *Effect of pH* : The medium was adjusted at different pH. The results are summarized in table 1.

TABLE 1

Showing growth and sporulation of *Alternaria tenuis* at different pH values

pH of the medium	Average dry weight of the mycelium (mg)	Sporulation
2.0	-	-
3.0	-	-
4.0	28.2	Poor
4.5	45.8	Fair
5.0	57.7	Good
5.5	67.1	Excellent
6.0	65.2	Excellent
6.5	52.8	Good
7.0	48.4	Good
7.5	40.9	Fair
8.0	33.7	Poor
9.0	20.4	Few spores

Average = 38.3

S.E. = 1.09

C.D. at 5% level = 3.1

The growth of *Alternaria tenuis* was obtained at all the pH except at pH 2 and 3. The pH 5.5 was the optimum for growth as well as sporulation. Growth and sporulation decreased as the acidity or alkalinity of the medium deviated from the optimum.



2. *Carbon nutrition* : The influence of twenty carbon sources on growth and sporulation of *Alternaria tenuis* was studied. Results are presented in table 2.

TABLE 2

Showing growth and sporulation of *Alternaria tenuis* on different carbon sources

Source of carbon	Average dry weight of mycelium (mg.)	Sporulation
L-Arabinose	46.0	Poor
D-Xylose	50.8	Fair
L-Rhamnose	40.6	Poor
D-Glucose	73.0	Excellent
D-Fructose	57.8	Good
D-Galactose	62.4	Poor
D-Mannose	52.0	Fair
Sucrose	71.0	Good
Maltose	64.6	Excellent
Lactose	62.4	Good
Raffinose	45.2	Excellent
Starch	53.3	Excellent
Dextrin	42.2	Excellent
Inulin	20.2	Excellent
Glycerine	16.2	Excellent
Dulcitol	25.0	Poor
Mannitol	42.0	Fair
Sorbitol	50.3	Excellent
Tartaric acid	00.0	-
Malic acid	32.2	Poor
Without any carbon source	00.0	-

Average = 43.2

S.E. = 1.02

G.D. at 5% level = 2.9

There was no growth in complete absence of carbon or on tartaric acid where the condition did not improve even by changing the pH. All other sources of carbon were utilized.

Xylose was a good source and it was best amongst the pentoses. Similarly all the hexoses and disaccharides were also good for growth. Raffinose supported moderate growth of the fungus. Starch, dextrin and inulin were good, moderate and poor sources respectively. The organism developed poor mycelial growth on glycerine and dulcitol but it was moderate and good respectively on mannitol and sorbitol. Malic acid induced significantly poor growth.

The table clearly indicates that sporulation of *A. tenuis* was greatly influenced by the source of carbon. *A. tenuis* recorded fair sporulation on xylose. It was thus a better source for growth as well as sporulation though the rest of the pentoses (arabinose and rhamnose) supported poor sporulation only. Amongst hexoses, glucose was the best sugar as it supported excellent sporulation. It was also excellent on maltose, raffinose, glycerine, sorbitol and on all the polysaccharides. Sucrose, lactose and fructose induced good sporulation while rest of the compounds were either fair or poor sources of carbon for sporulation.

3. *Nitrogen nutrition*: The influence of twentyfour nitrogen sources on growth and sporulation of the fungus was also investigated. The results are presented in table 3.

TABLE 3  
Showing growth and sporulation of *Alternaria tenuis* on different nitrogen sources

Source of nitrogen	Average dry weight of mycelium (mg.)	Sporulation
Potassium nitrate	66.1	Excellent
Sodium nitrate	55.6	Excellent
Calcium nitrate	57.0	Few spores
Magnesium nitrate	50.9	Excellent
Sodium nitrite	13.5	-
Potassium nitrite	19.9	-
Ammonium chloride	24.8	-
Ammonium nitrate	44.1	-
Ammonium sulphate	33.0	-
Ammonium carbonate	43.9	-
DL-Alanine	84.7	Poor
DL-Phenyl alanine	83.3	-
Glycine	46.4	Fair
DL-Valine	36.5	Few spores
DL-Leucine	34.8	Few spores
DL-Aspartic acid	85.2	Good
L-Glutamic acid	96.4	Poor
L-Histidine	35.1	Poor
L-Arginine	36.7	Few spores
L-Asparagine	51.7	Good
Acetamide	63.7	Good
Peptone	76.4	Excellent
Thiourea	21.8	-
Urea	23.7	Poor
Without any nitrogen source	-	-

Average = 47.7  
S.E. = 1.45  
C.D. at 5% level = 4.1

TABLE 4

Showing growth and sporulation of *Alternaria tenuis* on combinations of various carbon and nitrogen sources.  
 "A" denotes dry weight in mgs. and "B" sporulation

Carbon sources	Nitrogen sources									
	Peptone		Glutamic acid		Potassium nitrate		Arginine		Ammonium sulphate	
	A	B	A	B	A	B	A	B	A	B
Sucrose	89.5	Excellent	81.9	Fair	70.4	Good	48.2	Poor	38.6	Absent
Maltose	73.9	"	103.4	"	67.7	Excellent	51.8	"	38.2	"
Glucose	77.4	"	96.6	Poor	72.7	"	36.1	Few spores	32.1	"
Rhamnose	56.0	Good	67.3	"	42.5	Poor	27.7	"	22.5	"
Malic acid	46.8	Fair	50.0	Few spores	32.6	Few	20.9	"	19.1	"
Tartaric acid	20.3	Few spores	0.0	-	0.0	-	0.0	-	0.0	-

Average = 40.4  
 S.E. = 0.99  
 C.D. at 5% level = 2.8

Table 3 shows that except magnesium nitrate, all the nitrates supported good growth. Good growth of the organism was also obtained on acetamide, peptone, and on few amino acids like phenyl alanine, glutamic acid, aspartic acid, and alanine. Rest of the compounds were not very satisfactory as they supported either moderate or poor growth. It failed to grow in absence of any nitrogen source.

Sporulation also varied considerably. Nitrates in general were excellent sources except calcium nitrate which supported the development of few spores only. Excellent sporulation was also recorded on peptone. Nitrites, phenyl alanine and ammonium compounds failed to induce any spore production. Sporulation was poor on glutamic acid though it was best for growth. It was either absent or poor in all cases where the growth was poor.

4. *Different combinations of carbon and nitrogen sources*: The influence of six carbon and six nitrogen sources on growth and sporulation was studied. All possible combinations (36 in all) were used. Results are summarized in table 4.

From table 4, it is clear that maltose-glutamic acid combination was best for growth. Poorest growth was recorded on a combination of potassium nitrite and rhamnose. *A. tenuis* failed to grow on combinations of tartaric acid with any of the nitrogen compounds except peptone where it developed poor growth. There was no growth when organic acids were used in combination with potassium nitrite.

Ammonium sulphate in combination with all the carbon sources did not support any sporulation. Similar result was obtained when carbon sources except maltose were combined with potassium nitrite.

5. *Sulphur nutrition*: The influence of ten sulphur compounds (both inorganic and organic) was studied. Results are presented in table 5.

TABLE 5  
Showing growth and sporulation of *Alternaria tenuis* on different sulphur sources

Source of sulphur	Average weight of mycelium (mg.)	Sporulation
Magnesium sulphate	63.1	Excellent
Potassium sulphate	56.5	—
Ammonium sulphate	28.6	—
Zinc sulphate	0.0	—
Sodium bi-sulphate	45.2	Poor
Sodium bi-sulphite	35.6	„
Sodium thiosulphate	55.3	Few spores
Potassium persulphate	0.0	—
Thiourea	17.2	—
Cystine	45.8	Few spores
Without any sulphur source	0.0	—

Average = 31.6  
S.E. = 1.1  
C.D. at 5% level = 3.4

The present pathogen failed to grow in complete absence of sulphur or when zinc sulphate or potassium persulphate was used in media. Thiourea and ammonium sulphate supported poor and moderate growth respectively. The growth on all the other compounds was good.

Like carbon and nitrogen, sulphur sources also influenced sporulation. The present organism was incapable of sporulating on potassium sulphate, ammonium sulphate and thiourea while it developed only few spores on sodium thiosulphate and cystine. The sporulation was poor on others except on magnesium sulphate which supported excellent development or spores.

### Discussion:

It is clear from the above investigation that *Alternaria tenuis* can grow at different pH and on various substrates. It shows best growth and sporulation at pH 5.5. The pH of the leaf extract of tomato was within a range of 5 to 6. Thus the pH of the host tissue was quite favourable for its growth.

Carbon, nitrogen and sulphur compounds were found to be essential for growth as the present organism could not grow in the medium which completely lacked any of them.

Nutritional studies on fungi have shown that the utilization of a sugar is influenced by its molecular structure. The superiority of xylose over the other two pentoses (arabinose and rhamnose) appears to be connected with the differences in their molecular structure. Tandon and Gerwal (1954) reported that the above pentoses were poor sources of carbon for the growth of *Alternaria tenuis* isolated from apples, but Tandon and Srivastava (1958) as well as Thind and Gill (1961) observed good growth of *Alternaria tenuis* (isolated from wheat grains) and *A. brassicae* respectively on all these pentoses. The present results differed from all of them because only xylose supported good growth while the other two were moderate sources only.

Hexoses and disaccharides supported good growth of the organism under study. Similar results were obtained by Ghosh (1960) for *Alternaria tenuis* causing rot of the fruits of *Pyrus communis* ("Nakh"). The results differed from those of Tandon and Grewal (1954) who found that the hexoses and disaccharides were moderate sources for *A. tenuis* causing a rot of apples. They also differed from those of Tandon and Srivastava (1958) because according to them lactose and fructose were poor sources for *A. tenuis* isolated from wheat grains.

Inulin is not hydrolysed by enzymes which act on starch or on sucrose. Poor growth of *A. tenuis* (isolated from tomato) on inulin may be due to the non-availability of necessary enzymes which are responsible for its hydrolysis. Tandon and Grewal (1954) as well as Tandon and Srivastava (1958) also obtained poor growth of their organisms on inulin.

Most fungi appear to utilize sugars with greater facility than their corresponding alcohols. It was found to be true in the present study for mannitol and dulcitol which were moderate and poor carbon sources respectively. Tandon and Grewal (1954), however, found that mannitol and dulcitol were good carbon sources for *Alternaria tenuis* isolated from apples.

Though many fungi can utilize tartaric acid but *A. tenuis* isolated from tomato leaves failed to grow on it. In this respect it resembled *Alternaria brassicae* (Thind and Gill, 1961) and *A. tenuis* (isolated from "Nakh", Ghosh, 1960).

Studies on nitrogen nutrition indicated that nitrates of potassium, sodium, and calcium, were good sources for its growth but magnesium nitrate supported moderate growth only. The difference in growth observed on magnesium nitrate appears to be due to the influence of metallic ions. Unlike nitrates ammonium compounds were comparatively poor sources. Brian *et al.*, (1947) have explained that fungi which make limited growth on ammonium nitrogen do so because they are unable to synthesize adequate amounts of the necessary three, four, and five carbon keto acids. Tandon and Srivastava (1958) also obtained poor growth of *Alternaria tenuis* (isolated from wheat grains) on ammonium compounds.

Urea supported poor growth of the organism under study though it served as the best source of nitrogen for the growth of *Alternaria ricini* (Pawar and Patel, 1957).

*A. tenuis* isolated from tomato leaves was incapable of utilizing sulphur from zinc sulphate and potassium persulphate. Similar results were obtained by Agarwal and Ganguli (1960) for *Pestalotiopsis versicolor*. The present organism developed good mycelial growth on sodium bi-sulphite and, therefore, it differed considerably from *Pestalotia malorum*, *P. psidii* (Tandon 1950) and *Pestalotiopsis versicolor* (Agarwal and Ganguli, 1960) which failed to grow on it.

A critical study of the above tables indicates that in some cases there was a clear correlation between growth and sporulation. Certain compounds which were good for growth were also good or excellent sources of sporulation, *e.g.*, glucose, fructose, sucrose, maltose, lactose, starch, sorbitol, nitrates of calcium, sodium and potassium, acetamide, peptone, aspartic acid and magnesium sulphate.

Similarly sources which were poor for growth were poor for spore production also, *e.g.*, dulcitol, malic acid, urea and histidine. In certain other cases, however, sources which were good for growth supported poor sporulation, *e.g.* galactose, glutamic acid, alanine, sodium bi-sulphate and sodium bi-sulphite. Conversely sources which were poor for growth like inulin and glycerine supported excellent sporulation. Some of the compounds, *e.g.* nitrites of sodium and potassium, thio-urea, ammonium compounds, phenyl alanine and potassium sulphate failed to support any sporulation.

### Summary :

Effect of pH as well as of various sources of carbon, nitrogen and sulphur on growth and sporulation of *Alternaria tenuis* Auct. causing leaf spot disease of tomato has been studied. *A. tenuis* could grow on a wide range of pH but 5.5 was the optimum pH for its growth and sporulation. It failed to grow on pH 2 and 3.

Carbon, nitrogen as well as sulphur compounds were essential for growth because it failed to grow on medium lacking any of these sources. The growth was good on xylose (a pentose), all the hexoses and disaccharides. The other two pentoses arabinose and rhamnose supported moderate growth. *A. tenuis* failed to grow on tartaric acid.

Nitrates supported better growth than ammonium compounds. Poor growth of the organism was obtained on nitrites. Amino acids were not of equal value. Glutamic acid was best nitrogen source for growth. Different combinations of carbon and nitrogen sources affected growth as well as sporulation. Best growth of *A. tenuis* was obtained on maltose-glutamic acid combination. Rhamnose-potassium nitrite combination was poorest for its growth. Tartaric acid retained

its toxic property with all the nitrogen sources except with peptone where the growth of the organism was found to be poor.

Amongst sulphur sources, magnesium sulphate was best for growth as well as sporulation. Zinc sulphate and potassium persulphate did not support any growth.

Sporulation of *A. tenuis* varied considerably on different sources. Nitrites, ammonium compounds, phenyl alanine, potassium sulphate and thiourea failed to support any sporulation. In some cases good growth was associated with good sporulation but occasionally the sporulation was good even when the growth was poor or *vice versa*.

#### Acknowledgements :

Authors are grateful to Dr. J. C. F. Hopkins, Director, Commonwealth Mycological Institute, Kew, Surrey, England and to Dr. E. E. Butler, Assistant Plant Pathologist, Department of Plant Pathology, University of California, U. S. A., for the help they have given in identification and confirmation of the cultures of *Alternaria tenuis*. We also wish to express our thanks to Dr. K. S. Bilgrami for his helpful suggestions. The junior author is thankful to the Ministry of Scientific Research and Cultural Affairs, Government of India for the financial help which made this work possible.

#### Literature cited :

- Agarwal, G. P. and Ganguli, S. Nitrogen and sulphur requirements of *Pestalotiopsis versicolor* (Speg.) Steyaert. *Phyton*, **14** : 159-165, 1960.
- Asthana, R. P. and Hawker, L. E. The influence of certain fungi on the sporulation of *Melanospora destruens* Shear and of some other Ascomycetes. *Ann. Bot.*, **50** : 325-344, 1936.
- Brian, P. W., Curtes, P. J. and Hemming, H. G. Glutinosin : a fungistatic metabolic product of the mould *Metarrhizium glutinosum* S. Pope. *Proc. Roy. Soc. (London) Ser. B*, **135** : 106-132, 1947.
- Ghosh, A. K. M.Sc. Thesis, Allahabad University, 1960.
- Pawar, V. H. and Patel, M. K. *Alternaria* leaf spot of *Ricinus communis* L. *Indian Phytopath.*, **10** : 110-114, 1957.
- Rangaswami, G. and Sambandam, C. N. Comparative studies on some *Alternaria* species occurring on Solanaceous hosts. *Indian Jour. Agr. Sci.*, **31** : 160-172, 1961.
- Tandon, M. P. D.Phil. Thesis, Allahabad University, 1950.
- Tandon, R. N. and Srivastava, J. P. Studies on the spore characters of *Alternaria tenuis* Auct. *Sensu. Str. Proc. Nat. Acad. Sci. India*, **28** : 155-171, 1958.
- and Grewal, J. S. Studies of carbon selectivity from some organic compounds and its effect on growth and sporulation of *Alternaria tenuis* Strain B causing core rot of apples. *Proc. Nat. Acad. Sci. India*, **24** : 149-158, 1954.
- Thind, K. S. and Gill, P. S. Studies on the nutrition of fungi. V. The influence of the various carbon sources on the growth of *Alternaria brassicae* (Berk.) Sacc. *Proc. Nat. Acad. Sci. India*, **31** : 156-159, 1961.

# THE PHYSIOLOGY OF MIXED CROPPING

By

AMAR SINGH

Department of Botany, University of Allahabad, Allahabad

[Received on October 1, 1962]

Communications of Jagoe (1949), Melville and Sears (1953), Kenya Department of Agriculture (1954), Vicente-Chandler *et al* (1954), Hill and Moffett (1955), Harlan (1956), Masfield (1957), Varda Chari (1957) and Singh (1957, 1957a) are unanimous in depicting the economic advantages of mixed cropping of legumes and non-legumes in the tropic and the subtropic regions. Not unlike many questions, the problems related to symbiotic nitrogen fixation and mixed cropping between a legume and a non-legume yet remains unsettled. The excretion of nitrogenous compounds by legumes has been questioned by some and upheld by others (*cf.* Wilson, 1940; Russell, 1950; Whyte, *et al* 1953; Harlan, 1956).

The conceptions of the nature, amount and significance of the functions of legume root nodules have remained obscure; their relation to associated cultures has hardly received attention. The mere presence of nodules is not necessarily a criterion of optimum conditions resulting in adequate fixation of nitrogen in the soil. The *Rhizobia*, in the root nodules, are able to "fix" atmospheric nitrogen. Under optimal conditions nitrogen fixation may be rapid and continuous throughout the growing season whereas under adverse circumstances, the period of fixation may be transient and little nitrogen may be fixed. Symbiotic association between the host and *Rhizobia* in the nodules may thus be "effective" or "ineffective". With the introduction of a non-legume to grow in simultaneous rotation with the legume, in field practice, the circumstances change enormously and the study becomes still more complex.

In an earlier work, the associated growth of wheat and gram was demonstrated to be compatible for high yield and improved protein content (Singh 1957a), the increase in yield being governed among other factors, by seed rate proportionality of the companion crops (Singh, 1957). The ability of the cereal-legume mixture as between wheat and gram for better and more effective nitrogen fixation than the monoculture of legume was also established (Singh, 1954). The present studies were undertaken to elucidate if the resulting benefit of wheat (*Triticum aestivum*) and gram (*Cicer arietinum*) companionship may be traceable to the excretion of nitrogenous substances from the legume and whether the cereal partner could serve as an index of such an excretion. In addition, the extent to which wheat in association, may affect the process of excretion, fixation and transfer of combined nitrogen by the legume and also the influence of age and of developmental stage of the companion plants on nitrogen fixation under our subtropical conditions have also been assessed.

## Methods and Material :

### General :

Investigations were conducted in earthen pots of 18"×12" size filled with 8 Kg of sterilized and acid washed clay-free sand. 5 gm of soil from a field in which nodulated gram plants flourished was added to each pot to ensure the presence of the specific strain of *Rhizobium* effective on *Cicer arietinum*. Acclimatised



seeds of uniform size and absolute weight were used to avoid variations arising from influence of seeds of varying mass and relative density on dry matter production (Blackman, 1919). Seeds of *Triticum aestivum* (NP<sub>52</sub>) and *Cicer arietinum* (T<sub>97</sub>) were sterilized externally with 0.4 per cent solution of mercuric chloride for 4 minutes, washed clean in running water for 30 minutes and rewashed with sterilized distilled water.

Of the six seeds sown in each pot at a depth of 1.5 to 2.0 inches, three equidistant seedlings were allowed to grow, the other three were removed before the emergence of the first leaf in accordance with the recommendations of Whyte *et al* (1953), to avoid any nodulation and possible nitrogen fixation in the medium of growth. Nitrogen deficient culture solution of the following composition was autoclaved at 20 lb pressure for 20 minutes, kept overnight, and supplied at weekly intervals.

K <sub>2</sub> HPO <sub>4</sub>	- 0.50 gm	KH <sub>2</sub> PO <sub>4</sub>	- 0.50 gm
MgSO <sub>4</sub>	- 0.20 gm	NaCl	- 0.10 gm
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	- 2.00 gm	FePO <sub>4</sub>	- 0.50 gm
FeCl <sub>3</sub>	- 0.01 gm	Distilled water	- 1,000 cc

The treatment comprised of the following :

- (i) Fallow (unsown) pots with sterilized sand receiving regular and similar cultural treatments as applied to the cropped (sow) ones.
- (ii) Cropped pots with monoculture of gram alone,
- (iii) Cropped pots with monoculture of wheat alone,
- (iv) Cropped pots with associated culture of gram and wheat.

Each series consisted of six replicate of 15 pots. Random sampling was done from three pots of each replicate of a series, the observations recorded are presented on 'per plant' basis.

A couple of days before each sampling date the pots were marked out, at random, for sampling and were kept unwatered to facilitate the removal of sand without any loss of either the root system or sand. First sampling was carried out 30 days after the seeds were sown; plants were removed carefully, and the whole of the sand from each pot emptied separately. Sand from the pots was subsequently dried and detached root fragments, if any, were recovered by passing the sand through a sieve. All washings of the roots of culture plants thus sampled were returned to their original sand samples for correct accounting of nitrogen excretion.

#### Analytical :

Total nitrogen of sand was estimated after the method of Gunning and Hibbard with over six hours of digestion as recommended by Miller and Houghton (1945) and also Chibnal and Associates (1943). Kjeldahl's digestion method was employed for plant nitrogen determination (AOAC, 1945). Soil carbon was determined after the modified method of Walkley (Piper, 1947). Plant carbohydrate was estimated by the Hagedorn and Jansen's ferricyanide titration method modified by Sydney Cole (1933).

#### Nitrogen Excretion Determinations :

'Fallow' pots were maintained clean of vegetation in order to limit the factor of non-symbiotic nitrogen fixation, if any. These also served as a check of any increase of absolute nitrogen as a result of seasonal changes, addition of water,

or on other accounts. A known weight of the sand sample was subjected to extraction process. It was mixed with sulphuric acidified water of pH 4, filtered through glass wool under reduced pressure repeatedly washed and filtered, concentrated under reduced pressure at 40°C to a volume of 100 cc to facilitate chemical examination.

True value of gain in nitrogen due to gram culture was arrived at by making allowance for the nitrogen content of the sand medium in fallow pots at successive stages from those supporting gram alone. The extent of the effect of wheat on nitrogen gain was evaluated by determination of nitrogen gains of the associated culture pots and subtracting from it the gains occasioned by the monoculture of gram. For evaluating changes in the absolute value of nitrogen, results were computed for the entire amount of sand in the pots and then converted to 'per plant' basis.

Data have, throughout, been subjected to statistical analysis for correct estimate of the responses, S.E. and C.D. due to treatments have been computed and tabulated with the data.

### The Findings :

#### *Dry matter Accumulation :*

Measured in terms of dry matter accumulation, there was a progressive increase at successive steps of growth in both wheat and gram whether grown single or mixed. The cereal and the legume differed greatly in their responsiveness to the two cultural methods. There was positive evidence to show that wheat plants, in association, gained consistently and significantly in dry matter synthesis over single stand, to the disadvantage of gram plants which markedly suffered at the 30, 70 and 90-day growth stages due to the nutritional competition under mixed culture (Table 1).

TABLE 1  
Dry matter accumulation of wheat and gram plants under  
conditions of single and mixed cultures  
(gm/plant)

Age in days	Single culture		Mixed culture	
	Wheat	Gram	Wheat	Gram
30	0.442	0.790	0.561	0.495
50	0.701	0.898	2.300	0.972
70	0.782	1.348	2.740	1.041
90	0.873	1.487	2.800	1.190
Mean	0.6995	1.1307	2.000	0.9250
S.E. = 0.052                      C.D. = 0.1663				

The available nitrogen, whether from the sand or atmosphere, as fixed by the root nodules of gram, possibly, fell short of the actual requirements of the gram plants owing to its rapid intake by the wheat plants, growing alongside, for their advantage. Dry matter accumulation of gram plants under mixed culture was less as against single cropping; elaboration of dry matter at the 50-day age, though slightly higher, was not significantly so.

### Plant Nitrogen :

The mean nitrogen content of gram plants was significantly greater in single culture series than in mixed culture, especially so, at the fruiting stage (Table 2). Steady and larger increases in the nitrogen content of the legume, from seeding to maturity, was observed in pure as against mixed stand. Gram plants, in single culture, depicted a higher nitrogen percentage (mean) than those of wheat in pure stand, whereas in mixed cultures, the reverse was noted.

TABLE 2  
Nitrogen content of wheat and gram plants under conditions  
of single and mixed cultures  
(mgm/plant)

Age in days	Single culture		Mixed culture	
	Wheat	Gram	Wheat	Gram
30	5.40	4.50	6.80	3.90
50	7.40	10.70	23.60	9.70
70	10.40	17.34	39.40	12.02
90	13.60	35.50	42.00	14.92
Mean	9.200	17.010	27.950	10.135
S.E. = 3.347		C.D. = 10.71		

The effect of associated growth in inhibiting the accumulation of nitrogen in gram plants aggravated with age. Nitrogen of the associate wheat plants increased all through due to the association effect of gram ; the increase registered being of a higher magnitude than its loss from the associate gram plant at the corresponding stages in the life cycle. Nitrogen change in the wheat plants was maximum during the vegetative phase followed by that in flowering. At fruiting, there was actually less nitrogen accumulation in wheat plants of associated culture than of single, possibly, due to the fact that nitrogen in associated growth was utilized for more dry matter production in contrast to those in the single culture series.

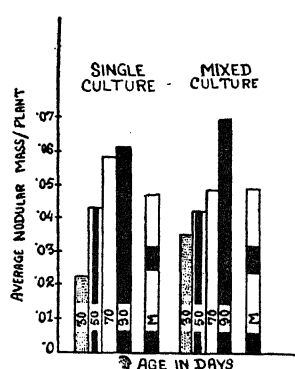


Fig. 1. The effect of Single and Mixed culture on Nodular Development during ontogeny of *Cicer arietinum*.

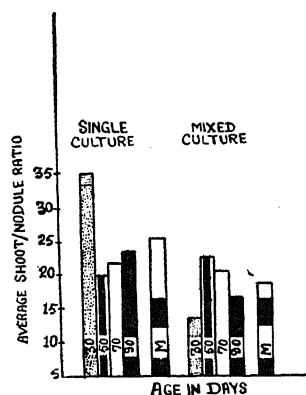


Fig. 2. The effect of Single and Mixed culture on Shoot-nodule ratio in the ontogeny of *Cicer arietinum*.

### *Nodule Production :*

Mixed culture as compared to single increased nodular mass at the 30 and 90-day age of the gram plants (Fig. 1). The effect of mixed culture on shoot/nodule ratio, on weight basis, was higher in single culture series throughout the life of the plant except at the 50-day age (Fig. 2). In general, mixed culture treatment failed to establish any significant effect on nodular mass, taken singly or in relation to the growth of the tops of the plant, though the effectiveness of such a nodulation was very much higher as evidenced by nitrogen excretion which continued throughout the life cycle of the plant (*cf.* Table 4).

### *Nodular Nitrogen :*

Nodular nitrogen increased with age irrespective of the mode of cropping. Significantly greater amount of nodular nitrogen was found under conditions of double culture than in single throughout the ontogeny of the gram plant.

TABLE 3  
Nodular nitrogen in gram plants under conditions of single and mixed cultures  
(mgm/plant)

Age in days	Single culture	Mixed culture
30	1.00	1.30
50	1.40	1.90
70	1.64	2.12
90	2.50	2.62
Mean	1.64	1.99

S.E. = 0.05

C.D. = 0.2250

Mixed culture consistently increased nitrogen of the nodule, the benefit over single culture was progressively marked upto the 70-day age after which it narrowed (Table 3).

### *Nitrogen Excretion :*

Consistent surplus nitrogen excretion by gram, obviously, showed that its excretion was faster than utilization. The variations in nitrogen excretion could only occur with ontogenic drifts in the growing capacity of the legume at successive stages in the life cycle. The associated culture was marked by consistent increase in the amount of nitrogen excreted into the medium in excess of single culture by 14.75, 38.30, 63.80 and 87.30 mgm at the age of 30, 50, 70 and 90 days respectively (Table 4).

TABLE 4  
Excretion of nitrogen by gram plants under conditions of single and mixed cultures  
(mgm/plant)

Age in days	Single culture	Mixed culture
30	36.85	51.60
50	39.40	77.70
70	54.20	118.00
90	40.70	128.00
Mean	42.790	93.825

S.E. = 11.09

C.D. = 59.90

Stage to stage increase or decrease in the extent of excretion in the two cultures and the superiority of mixed culture over single could be clearly made out. In both single and mixed cultures nitrogen excretion (Table 4) and nitrogen fixation (Fig. 3) was at its peak at flowering.

TABLE 5

Combined transfer of nitrogen by gram plants under conditions of single and mixed cultures (mgm/plant)

Age in days	Single culture	Mixed culture
30	40.35	54.20
50	48.70	85.50
70	69.90	127.91
90	74.20	140.30
Mean	58.29	101.98
S.E.	= 8.779	
C.D.	= 37.25	

Unlike nitrogen, excretion transfer for combined nitrogen and also real fixation increased steadily with age, maximum increase being recorded during the reproductive phase (Tables 5 and 6).

TABLE 6

Real fixation of nitrogen by the gram plant under conditions of single and mixed cultures (mgm/plant)

Age in days	Single culture	Mixed culture
30	41.35	55.50
50	50.10	87.47
70	71.54	130.03
90	76.28	142.90
Mean	59.797	103.957
S.E.	= 8.319	
C.D.	= 37.43	

Expressed as per cent of real fixation the amount of nitrogen excretion was highest during plants' early stages, declining with age. The value for mixed cultures was 92.96 per cent during the juvenile stage which fell down to 89.85 per cent at the close of the life cycle; in the single culture series the values of the comparable stages stood at 89.08 and 53.34 per cent respectively depicting a steep fall towards maturity.

#### *Fixation and Translocation of Nitrogen :*

Nitrogen present in the nodules was lower than that in the plant under the two cropping conditions showing that the process of nitrogen fixation and its trans-

location from the nodules continued almost simultaneously. The ratio of nitrogen transferred to that fixed decreased (not the rate) during flowering under the two cultural conditions. The ratio remained higher in single culture (66.28, 29.85 and 381.97 at vegetative, flowering and fruiting respectively) in comparison to mixed culture (16.04, 4.50 and 18.63 at vegetative, flowering and fruiting respectively).

In both the cultures a sharp rise in the nitrogen fixed during flowering was accompanied by a steep fall during fruiting (Fig. 3). The rate of transfer of nitrogen to the soil was of a higher order in single culture than in associated, throughout the life cycle where the rate of fixation was high with the result that more nitrogen was fixed in mixed than in mono-culture.

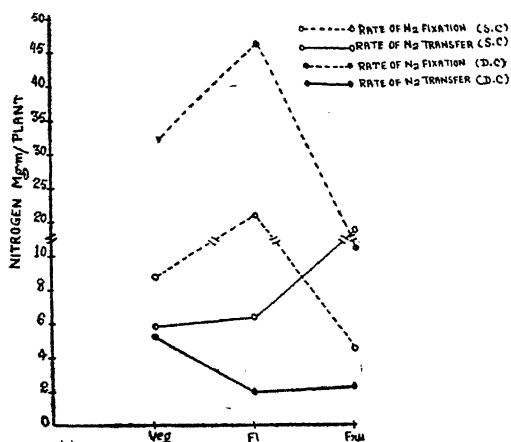


Fig. 3. The effect of Single and Mixed culture on the rate of fixation and the rate of nitrogen transfer during the ontogeny of *Cicer arietinum*.

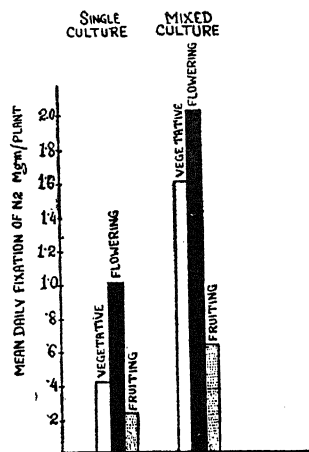


Fig. 4. The effect of Single and Mixed culture on nitrogen fixation by *Cicer arietinum*.

#### Real Fixation and Combined Transfer of Nitrogen:

Real fixation<sup>1</sup> and combined transfer<sup>2</sup> of nitrogen values remained greater in associated cultures alike that of nitrogen excretion (*cf.* Tables 5 and 6). The rate of real fixation of nitrogen was higher in mixed culture than the rate of combined transfer throughout the ontogeny of the gram plant.

1. *Real fixation* denotes the nitrogen content of the host plant inclusive of that present in the nodules *plus* the amount excreted in the medium in the case of the single culture ; in the associated cultures, however, the amount of nitrogen taken up by the cereal is also added.
2. *Combined transfer* denotes the nitrogen content of the host plant exclusive of that in the nodules *plus* the nitrogen excreted into the medium. In associated cultures the values of excreted nitrogen also includes the amount of nitrogen transferred to the companion crop.

TABLE 7  
Real fixation and combined transfer by gram plant as affected  
by the cropping conditions

Physiological stage (days)	Single culture			Mixed culture		
	Rate of fixation	Rate of combined transfer	Ratio	Rate of fixation	Rate of combined transfer	Ratio
Vegetative (30-50)	8.75	8.35	95.42	31.97	31.30	97.90
Flowering (50-70)	21.44	21.20	98.88	42.56	42.41	99.64
Fruiting (70-90)	4.74	4.30	90.71	12.87	12.40	97.04

Ratio = 100 (Rate of combined transfer/Rate of fixation)

In single culture, however, rate of combined transfer did not show marked differences over the rate of real fixation upto the flowering period, during the fruiting stage both combined and real fixation exhibited significant decline (Table 7). A vigorous fixation by the gram plant grown in association with wheat was also depicted.

A close parallelism was shown to exist between combined transfer and fixation of nitrogen (*cf.* Tables 5 and 6) throughout the life cycle of the legume; a point clearly brought out by the ratio of nitrogen transferred/nitrogen fixed. Nodules seemed to part with considerable portion of the symbiotically fixed nitrogen regularly to the host plant and to the medium of growth.

#### *Efficiency of Real Fixation :*

Efficiency of real fixation, as calculated after the formula suggested by Bond, (1936), increased with age and was significantly greater in mixed culture (Table 6). Maximum fixation occurred during flowering in both the cropping schemes. The mean dry weight of the nodules (Fig. 1) increased with age in both the cultural series, though fixation was not proportionate showing, thus, that the efficiency of nitrogen fixation was not totally a function of the absolute mass of the nodular tissue. The rate of real daily fixation of nitrogen by gram was found to be significantly more in mixed culture series than in single (Fig. 4).

#### *Gram-Wheat Association :*

Changes in dry weight and nitrogen content of wheat under conditions of single and mixed culture showed the performance of the gram on the two accounts. The difference in dry matter accumulation in wheat as between single and double culture was greater towards the close of the life cycle, showing thereby that wheat continued to grow and augment dry matter production in the company of gram till a later stage. The rate of such an augmentation being greater in mixed culture upto the flowering stage though in the fruiting stage the mono-culture exceeded (*cf.* Table 1).

That the companion cereal utilised adequate quantity of nitrogen out of the excreted nitrogen from the root nodules of the legumes to meet its growth requirements was evindenced by the high nitrogen content (42.00 mgm) in the mature wheat plant grown in association with gram, compared to 13.60 mgm in single culture series (Table 2). Wheat, in association with gram gained both in dry weight and nitrogen at all stages in the life cycle of the plant, the maximum gain being at the pre-flowering stage.

The companion legume plant contained significantly less nitrogen (14.92 mgm) as compared to its pure stand (35.50 mgm). This explains for the slower rate of nitrogen excretion from root nodules of the single culture gram; real fixation value of nitrogen was almost double in the case of associated culture, in all probability, owing to the fact that the legume was deprived of its nitrogen by the associated non-legume. The overall effect of cereal-legume association on the legume has been depicted below (Table 8).

TABLE 8  
The overall effect of cereal legume association on the  
companion legume

Characters	% increase or decrease	
Dry matter (gm, tops)	—	17.76
Nitrogen (mgm/plant)	—	40.41
Nitrogen (mgm, nodule/plant)	—	31.78
Nódular mass (gm/plant)	+	4.34
Shoot-nodule ratio (per plant)	—	27.82
Nitrogen excretion (mgm/plant)	+	119.28
Combined transfer of nitrogen (mgm/plant)	+	74.90
Real fixation (mgm/plant)	+	73.84

Both fixation and excretion of nitrogen by the legume increased with age; the relative pace of the two processes experienced a gradual rise in single culture series with advance in age, which indicated that under normal conditions larger and larger share of fixed nitrogen was utilized by the gram plant for anabolic processes. The correlation between fixation and excretion was of a higher order in plants of associated culture than in single culture.

#### Discussion :

Arable soils lose, progressively, nitrogen and therefore there must be some natural means to offset these losses if its crop producing power has to be maintained. Besides crop rotations, mixed cropping between a cereal and a legume provided considerable gains to the soils and also to the cereal grown in association.

Associated growth of wheat and gram led to augmentation of dry matter accumulation and also in plant-nitrogen of the cereal component while in the case of the legume component there followed an increase in the nodular mass, shoot/nodule ratio, nodular nitrogen, nitrogen excretion, combined transfer and real fixation of nitrogen. Adequate legume-nonlegume partnership between gram and wheat was shown to increase nodulation significantly (Singh, 1957a), though insignificantly in the present case. This may be due to the seed rate proportionality



factor being in operation (Singh, 1957). Scientific evidence has been put forward to show that nodulation in legumes is controlled by additives (Singh, 1958), clipping of shoots (Singh, 1958a), daylength (Singh, 1958b) and the nature of the legume, whether raised for seed or forage, (Singh, 1958c) under mono-culture conditions.

Mixed culture increased dry matter accumulation in wheat by 1.48, 0.36 and 0.06 gm during the vegetative, flowering and fruiting stages, respectively, though nitrogen changes at comparable physiological stages were to the tune of +14.80 mgm, +12.80 mgm and -2.60 mgm. Increase in the plants' nitrogen content between any two physiological stages was 3 to 3.2 mgm for single culture while in mixed culture it was as high as 16 mgm (Table 1). The trends of dry matter production and nitrogen accumulation in wheat grown singly and mixed were not exactly identical; losses of nitrogen from the associate wheat plants at fruiting provided the clue to its possible exudation.

Earlier, La Flize (1892), Lyon and Bizzell (1911), Lipman (1912), Jagoe (1949), Sears (1953), Singh (1954, 1957a) reported that legume may supply nitrogen to a nonlegume. Legume-nonlegume partnership in the utilization of nitrogen has been reported to be more beneficial than rotation by Hill and associate (1955). These and other findings point to the existence of some mechanism by which the legume enriches the soil in nitrogen possibly through its excretion in organic combination.

TABLE 9  
Effect of mixed culture on Fixation, Translocation and Combined  
Transfer of nitrogen from gram plants  
(mgm/plant)

Physiological stages (days)	Fixation	Translocation	Combined transfer
Vegetative (30-50)	+ 23.65	- 0.60	+ 22.95
Flowering (50-70)	+ 25.18	- 4.30	+ 21.21
Fruiting (70-90)	+ 8.22	- 15.40	- 29.91

The effect of the associated culture remained almost consistently positive so far as nitrogen excretion was concerned (Fig. 5). Increase in the amount of combined nitrogen of the medium of growth decreased the efficiency of nitrogen fixation by gram (Singh, 1952) while the reverse condition, *i.e.*, decrease in soil nitrogen through nitrogen intake by the associate nonlegume, increased, excretion and fixation of nitrogen. Mixed culture conditions increased excretion of nitrogen (Table 4).

There was positive evidence to show that increase in the dry matter production of the competing wheat plant was due to utilization of nitrogenous compounds synthesised by the associate legume. Russell's contention (1950) that such a benefit could be interpreted as showing that the legume made no demands on the soil

nitrate and hence the reduced number of nonlegumes present in association, as compared with pure stand, had a larger supply of nitrogen to draw on, cannot be held valid, under the conditions of these experiments, since no nitrogen was supplied to the medium and observations made on the check pots with no culture, single culture, and double culture have all been adequately accounted for in arriving at the results.

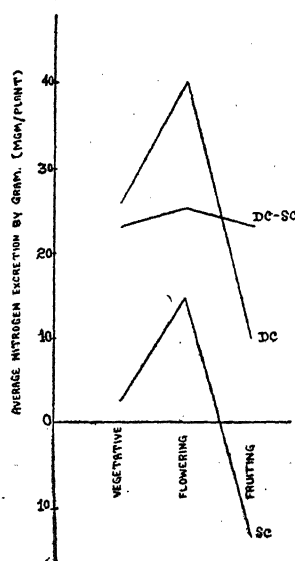


Fig. 5. The effect of Single and Mixed culture on excretion of nitrogen by *Cicer arietinum*.

Horst Borner (1960) has reviewed the literature pertaining to excretion from intact roots, but has not considered the association effect specially between a legume and cereal. The evidences put forward herein, supported by definite response of companion wheat, and mustard in another series of investigations (Singh, 1957), provided the basis to substantiate the earlier but questioned findings of Thatcher (1925), Hackleman and Collaborators (1928), Odland (1930), Nicol (1936), Virtanen (1938) and Demidenko and Timofeeva (1937) in respect of nitrogen excretion. Conflicting evidences were put forward by Bjalfve (1935), Ludwig and Allison (1937) and Nowotownna (1937) who obtained proof of excretion only in a limited number of case while Scholz (1939) and Madhok (1940) reported excretion by legumes but failed to establish the benefit of such an excretion by companion nonlegume. Virtanen *et al* (1936) reported upon the excretion of lysine and aspartic acid by root nodules of leguminous plants. Wilson (1932) failed to secure evidence of excretion of nitrogenous compounds by legumes either when grown alone or with a cereal. Uniformly negative results in respect of excretion reported by Wilson and Burton (1938), Engel and Roberg (1938), Bond (1938), Shapter (1939) and Bond and Boyes (1939) stand negativated by experimental results herein reported. The present findings stand in contradistinction to those reported by Trumble and Strong (1937) who, among others, failed to record appreciable evidence of excretion of nitrogen during the vegetative growth of a legume. Excretion was shown to occur at the vegetative, flowering and fruiting stages in both single and mixed culture series suggesting that nodulation, as defined

by Thornton (1939), was "effective" and that the present circumstances helped both nodulation and nitrogen fixation.

Decline in excretion and fixation of nitrogen during fruiting may appropriately be ascribed to the reabsorption of a portion of the excreted nitrogen in the rapid translocation that followed to supply the growing need of the developing gram seeds. Any valid estimate of the absolute amount of the excreted nitrogen could not be done as the process of reabsorption by the plants was beyond experimental control.

A close correlation between excretion and real fixation could be traced as the rate of excretion varied directly with that of the fixation, barring the slight discrepancy in single culture. (Tables 4 and 6). Evidence of excretion also exist in the case of sugarcane (Ranjan *et al.* 1952), while fixation of nitrogen by nonlegumes has been reported by many sources (*cf.* Shields, 1953, Wilson, 1940). That wheat in association with gram is able to fix nitrogen to a greater extent than when grown singly is also known (Singh, 1954). Addition of nitrogenous fertilizers augment nitrogen accumulation by the wheat plant (Singh, 1942); axiomatically it is more likely, than not, that the legume grown as companion crop also increased the nitrogen supply to the associate cereal. Excretion or fixation of nitrogen by the wheat plant with consequent increase in soil nitrogen has been recorded (Singh, 1942); the greater the nitrogen in the plant, the greater was the extent of excretion leading to a narrow C/N ratio.

The process of excretion, transfer of combined nitrogen and its fixation seemed to be regulated by the conditions of growth to which the plants were subjected; conditions of associated culture afforded better facilities to the transfer of nitrogen leading to greater excretion. The rate of transfer of nitrogen in the plants was higher when gram was grown singly; it increased with age although reverse was true in the case of mixed culture (Fig. 3). The ratio of nitrogen transferred to nitrogen fixed was maximum at the fruiting stage in both the series (381.97 for single culture and 18.63 for double culture). Single culture presented a higher ratio at different physiological stages as compared to their counterpart in mixed cropping (vegetative stage: 66.28 and 16.04; flowering stage: 29.85 and 4.50 for single and mixed cropping). The flowering stage, the period of maximum excretion, depicted maximum fixation, least transfer and maximum ratio  $\left( \frac{100 \times \text{rate of transfer}}{\text{rate of fixation}} \right)$  under the two cropping conditions (Fig. 3).

The benefit in plants of the double cropping series may be due to excretion brought about by the disturbed equilibrium of one or more of the factors such as the stimulating effect of wheat on the activity of the *Rhizobia*, changes in mineral status, soil pH and excretion by the wheat plants itself. Greater nitrogen excretion by the gram plants was detected when the rate of its utilization fell short; the surplus nitrogen excreted was either taken up by the companion wheat or left in the soil. The amount of nitrogen present in the medium of growth and transferred to wheat was much higher than that taken up by the legume itself.

In mixed culture series the gram plant, acting as a liberal host, suffered heavily as it could make use of but a small fraction of the fixed nitrogen and was prompted to part with larger amount of nitrogen to the companion wheat. Lower dry weight accumulation of gram plants grown in association with wheat showed that the carbohydrate utilization was slower in monoculture. The explanation offered by Wilson (1940) that bacteria in the nodules need a supply of energy, as carbohydrate, if these have to fix nitrogen, seemed to be relevant and are supported by the present findings. Fixation of nitrogen by the plants from the very early stages indicated that fixation of nitrogenous substances by the *Rhizobia* took place,

possibly, during the process of bacterial respiration with the utilization of carbohydrates of the host plant since only then the release of a large amount of nitrogen either to the host or to the surrounding medium, could be explained.

Wheat plant acted as a perfect indicator of the excretion of nitrogen by the root nodules of the gram plant. In single culture, the process of excretion seemed to be favoured by a high rate of fixation relative to growth and the existence of an equilibrium determining the distribution of nitrogenous compound between the nodules and the surrounding medium of growth. In the associated culture of gram and wheat the equilibrium shifted much further on the side of the medium owing to the ability of the wheat plants to absorb continuously and simultaneously the excreted nitrogen. As the carbohydrate synthesis did not keep pace with nitrogen fixation, the plant could not, obviously, make advantageous use of the nitrogen fixed towards rapid formation of new tissues.

The data further provide evidence to show that as active *Rhizobia* in the root nodule arrest more atmospheric nitrogen than the host plant could metabolise the excess excreted into the soil may enhance production of carbon dioxide by favouring bacterial activity as advocated by Demolon (1947). The surplus nitrogenous compounds accumulated in the nodules, lying in contact with highly absorptive surfaces of the medium and the root of the companion crops was, therefore, transferred with large increase in the rate of carbon assimilation, the rate of excretion of the nitrogenous compounds from the root decreased; in fact, during advanced seedling stage of the plant reabsorption of nitrogen from the substrate could not be ruled out pointing to the intimate relationship and mutual partnership between carbohydrate supply by the host and the nitrogen of the bacteria. The growth of the wheat plants in association with gram induced a change in photo conditions, temperature, and moisture such that photosynthesis in the legume could not out-distance the assimilation of free nitrogen resulting in restricted utilization of the latter to enable its excretion.

Conditions which opened up possibilities of greater excretion by legume were detected to be necessarily ill-conducive to its transfer in the plant body and *vice-versa* (Tables 2 and 3). The nitrogen content of the gram plant being higher in single culture than that in the mixed and that of the wheat plants having a reverse trend was, thus, amply justified by the physiological behaviour of the two plants. Excretion seemed to be a mechanism by which a favourable carbohydrate-nitrogen balance was maintained in the plants. The readjustment of the grain : straw ratio in the gram under the different wheat-gram mixtures (Singh, 1957), lends further support to the existence of such a controlling influence in the associated growth of cereal and legume.

The efficiency of fixation of nitrogen increased, with age, irrespective of the cultural conditions. Mixed culture did increase mean daily fixation of nitrogen as against single culture (Fig. 4). The maximum mean fixation by the *Rhizobia* coincided with the flowering stage of the host plant and declined thereafter (Fig. 4). A reduction in the mean daily fixation of nitrogen by the single and associated cultures of gram might be attributed to the enhanced demand for available carbohydrate between the host and the *Rhizobia*; it might also be due to the passing of bacteria into inactive bacteroid form, during the latter stages, as suggested by Bazarewski (1927). The efficiency of real fixation was not proportional to the nodular mass at the different stages though the two increased with the age. Mixed culture conditions increased both fixation and combined transfer but not translocation of nitrogen (Table 10), which pointed out that the two sets of processes could not be related positively.

The flowering stage of wheat corresponded with the period of maximum nitrogen fixation when the cereal was able to deprive the legume of its nitrogen to maximum extent, a finding that is supported by the work of Wilson and Wyss (1941). When gram was in flower, grain formation in wheat continued and this favourable parallelism between the life cycles of the two companion crops helped wheat to absorb adequate amounts of nitrogenous compounds at the expense of the associated legume whose yield was definitely lowered in partnership; the wheat plant thus served as an excellent index of the excretion of nitrogen by gram plant. It was shown earlier (Singh, 1957a) that mustard was not a profitable companion for gram, possibly, since the time of maximum nitrogen excretion did not coincide with the period of highest demand of nitrogen by the mustard which flowers and ripens earlier.

TABLE 10  
The effect of the wheat-gram association on wheat plants  
(DC—SC)

Physiological stages (days)	Dry matter gm/plant	Plant nitrogen mgm/plant
Vegetative (30-50)	+ 1.48	+ 14.80
Flowering (50-70)	+ 0.36	+ 12.80
Fruiting (70-90)	+ 0.06	- 2.60

The suitability of the companion crops, thus, depended on their biochemic constitution and elemental requirement during the ontogeny. Benefit accrued to associated wheat (Table 10) might well be ascribed to the interpenetration of roots which facilitated rapid secretion and transfer of combined nitrogen by the companion legume as also held by Thornton (1935). In case the rooting habit of the companion crops, as in mustard and gram, were at variance such an association proved unproductive (Singh, 1957a). The experimental evidences, thus point to the advisability of growing only such crops mixed as have a close parallelism in the physiological course of their growth, development, rooting habit and nitrogen requirement, on the one hand and the excretion of nitrogen by the legume coinciding with the need of the non-legume associate, on the other.

The relationship between the *Rhizobia* and its host was, thus not of a purely chemical nature neither it did rest on the external factors in complete disregard of the internal ones that controlled the mechanism *in vivo*. The rate of fixation varied with reason, and the physiological stage of growth of the host. Under normal and favourable conditions the fixation of nitrogen was many times greater than that present in the nodules or that recovered by the host. Three distinct phase of the fixation of the nitrogen thus existed coinciding with the vegetative, flowering and fruiting stages of the plant controlled partly by carbohydrate supply position.

The existence of relationship between the adjustment of carbohydrate-nitrogen balance leading to the changeover from vegetative to the developmental

phase of the plant on the one hand and nitrogen fixation on the other is indicated. It is abundantly clear that fixation presented a truly cooperative function of the two organisms – the *Rhizobia* and the host ; the host supplied carbohydrates to the bacteria to be utilized for energy and the bacteria in its turn supplied nitrogen. To increase nitrogen fixation it was essential that the nitrogen of the host was removed away as fast as possible from the plant so that maximum energy in the form of carbohydrates could be utilized by the host ; gram-wheat association provided the needed conditions in this respect.

#### Summary :

In the associated growth of the wheat and gram in equal stand, on seed weight basis, with well washed sterilized sand as medium of growth under relatively controlled pot culture trials, the effect of the cereal companion on nodule characteristics as well as, excretion, transfer and fixation of nitrogen by the legume has been investigated.

An enquiry has been made to elucidate if the resulting benefit of gram-wheat companionship could be traced to the excretion of nitrogenous substances from the legume and whether the cereal partner could act as the index of such an excretion. Assessment has also been done as to how far wheat, in association, may affect the process of excretion, fixation and transfer of the combined nitrogen by the legume and also of the influence of age and developmental stages of the plant on the fixation of nitrogen by the legume.

Nodular nitrogen, nitrogen excretion and nitrogen fixation were of higher order in gram grown mixed with wheat. Nodular growth and activity could be gauged by the excretion or fixation of nitrogen. Wheat plants served as a perfect index of the measure of the excretion of the nitrogen by the companion gram due to the fact that the period of maximum excretion of nitrogen coincided with that of the maximum demand by the companion cereal. This similarity in the ontogeny of the plant seemed to be the palusible governing factor for maximum profit in mixed cropping. When grown mixed, nitrogen recovery by the wheat plant was significantly larger while that of the gram plant less. The rate of the recovery of nitrogen by the legume and its fixation were inversely related ; while the former was appreciably lower, the latter was high in double culture. In single culture of the legume the position was much different. Nitrogen excretion and efficiency of its fixation rose upto pre-flowering stage and fell with the onset of the reproductive primordia. The efficiency of real fixation increased with age and was significantly accelerated in mixed cultures through internal adjustment in the legumes as well as change in the micro-climate.

The relationship between the *Rhizobium* species and the leguminous plant was shown to be not of an exclusively chemical nature as it did rest on the external as well as internal factors occasioned by the conditions of growth as well as developmental stages of the plant.

The mechanism of control of nitrogen fixation lay in making available, therefore, carbohydrates as a source of energy to bacteria after fulfilling the needs of the plant since mere presence of carbohydrates in the plant was not enough if it was not readily available for the symbiont. Nitrogen fixation by a legume should be considered with polyculture as a factor.

The *Rhizobia* inhabiting the root nodules of the gram plant needed a supply of energy in the form of carbohydrates from the host, the soil, or the companion crop. The associated crop created conditions conducive to better excretion of nitrogen.

The carbohydrate-nitrogen balance of the host plant was shown to be the chief *pivot* for the control of nodulation, symbiotic nitrogen fixation and related activities of the *Rhizobia* inhabiting the root nodules of the legume.

The advisability of growing only such crops mixed as have a close parallelism in the physiological course of their growth, development, rooting habit and nitrogen requirement on the one hand and the excretion of nitrogen by the legume coinciding with the corresponding need of the non-legume component on the other has been stressed. It has opened new line of thought on the role of mixed cropping on nitrogen recovery and recuperation.

#### Literature Cited :

- AOAC. Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Washington, D.C., 1945.
- Bazarewski, S. *Recz. Nauk. Rolnicz.*, **XVIII**, : 1-34, 1927.
- Bjalfve, G. 1935. Cf. Wilson: The Biochemistry of Symbiotic Nitrogen Fixation, 1940.
- Blackman, V. H. *Ann. Bot.*, **33** : 353-360, 1919.
- Boud, G. *Ann. Bot.*, **50** : 559-578, 1936.
- *Ann. Bot.*, n.s. **2** : 61-74, 1938.
- and Boyes, J. *Ann. Bot.*, n.s. **3** : 901-914, 1939.
- Borner, H. *Bot. Rev.* **XXVI**, **3** : 393-424, 1960.
- Chibnal, A. C., Rees, M. W. and Williams, E. F. *Biochem. Jour.*, **37** : 354, 1943.
- Demidenko, T. T. and Timofeeva, E. F. *Compt. Rend. Acad. Sci.*, (U. S. S. R.), **14** : 231-233, 1937.
- Demolon, A. Biol. Abst. No. 18199, 1947 (Original in *Compt. Rend. Acad. Sci.*, (Paris) **223** : 1076-1078, 1947).
- Engel, H. and Roberg, M. *Ber. Deut. Bot. Gesell.*, **56** : 337-352, 1938.
- Hackleman, J. C., Sears, O. H. and Burlison, W. L. *Ill. Agr. Expt. Sta. Bul.* **310** : 467-531, 1928.
- Harler, C. R. The culture and Marketing of Tea, London, Oxford University Press, 1956.
- Harlan, J. R. Theory and Dynamic of Grassland Agriculture, D. Van Nastrand Co., 1956.
- Hill, J. F. R. and Moffett, J. P. Tanganyika—A review of its Resources and their Development, Govt. of Tanganayika, 1955.
- Jagoe, R. B. *Malay. Agric. Journ.*, **32** : 77, 1949.
- Kenya Deptt. of Agriculture, Annual Report, Vol. II, 1954.
- La Flize, S. *Ann. Sci. Agron.*, **9** : 174-178, 1892.
- Lipman, J. G. *N. J. Agr. Expt. Sta. Bul.*, **253** : 48, 1912.
- Lyon, T. L. and Bizzell, J. A. *N. Y. (Cornell) Agr. Expt. Sta. Bull.*, **294** : 365-374, 1911.
- Ludwig, C. A. and Allison, F. E. *Jour. Amer. Soc. Agron.*, **27** : 895-902, 1935.
- Madhok, M. R. *Soil Sci.*, **49** : 419-432, 1940.
- Masfield, G. B. *Emp. Jour. Expt. Agrtc.*, **25** : 139, 1957.

- Melville, J. and Sears, P. D. *N. Z. Jour. of Sc. and Tech.*, **35A** Suppl. 1-30, 1953.
- Miller, L. and Houghton, J. A. *Jour. Biol. Chem.*, **159** : 373, 1945.
- Nicol, H. *Monthly Bul. Agr. Sci. and Practices*, **6** : 201-216, 1936.
- Nowotownna, A. *Jour. Agr. Sci.*, **27** : 503-510, 1937.
- Odland, T. E. *W. Va. Agr. Expt. Sta. Bul.*, **227** : 24, 1930.
- Piper, C. S. *Soil and Plant Analysis*, Adelaide, 1947.
- Ranjan, S., Singh, A. and Singh, U. S. Annual number, *Nat. Acad. Sci.*, 1952.
- Russell, E. J. *Soil conditions and plant growth*, Longmans Green & Co., 1950.
- Scholz, W. *Bodenk., Pflanzenernahr.*, **15** : 47-73, 1939.
- Sears, P. D. *N. Z. Jour. of Sc. and Tech*, **35A**, Suppl. 1, page 1, 1953.
- Shapter, R. E. *Jour. Council Sci. Ind. Res.*, **12** : 23-36, 1939.
- Shields, L. M. *Bot. Rev.* **XIX**, **6** : 321-376, 1953.
- Singh, A. M.Sc. Thesis, Inst. Agr. Res., B. H. U. (Unpublished), 1942.
- *Proc. Nat. Acad. Sci.*, Annual Number, 1952.
- *Bull. Bot. Assoc. Alld. Uni.*, **1** : 33-37, 1954.
- *Proc. Nat. Acad. Sci.*, **27B** : Part IV, 163-170, 1957.
- *Ibid.*, **27B** : Part IV, 180-185, 1957a.
- *Ibid.*, **28B** : Part I, 81-89, 1958.
- *Ibid.*, **28B** : Part II, 179-184, 1958a.
- *Ibid.*, **28B** : Part III, 231-241, 1958b.
- *Symp. Rec. Adv. in pl. Met.*, 113-117, Univ. Alld., 1958c.
- Sydney Cole, *Biochem. Jour.*, **27** : 3, 723, 1933.
- Thatcher, L. E. *Ohio Agr. Exp. Sta. Bull.*, **384** : 33-68, 1925.
- Thornton, H. G. *Third Intern. Congr. Soil Sci. Trans.*, **2** : 31-94, 1935.
- *Ibid.*, 20-23, 1939.
- and Nicol, H. *Jour. Agri. Sci.*, **24** : 269-282, 1934.
- Trumble, H. C. and Strong, T. H. *Austr. Coun. Sci. and Ind. Res. Bull.*, **105** : 11-24, 1937.
- Varda Chari, K. *World Crops*, **9** : 33-37, 1957.
- Vicente-Chandler, J., Caro-Coster, R. and Figarella, J., *Agron Jour.*, **45** : 397, 1954.
- Virtanen, A. I., T. Laine, and Hausin, S. V. *Nature*, **137** : 277, 1936.
- Virtanen, A. I. *Cattle fodder and Human nutrition*, Cambridge University Press, London, 1938.
- Wilson, P. W. and Wyss, O. *Soil Sci.*, **52** : 15-23, 1941.
- Whyte, R. O., Nilson-Leissner, G. and Trumble H. C. *Legumes in Agriculture*, F. A. O., Publication, 1953.
- Wilson, A. L. *N. Y. Cornell. Agr. Expt. Sta. Mem.*, **145** : 59, 1932.
- Wilson, P. W. and Burton, J. C. *Jour. Agri. Sci.*, **28** : 307-323, 1938.
- Wilson, P. W. *The Biochemistry of Symbiotic Nitrogen Fixation*, Madison 1940.



EFFECT OF NITROGEN STARVATION ON MULTIPLICATION  
OF ACTIVE TOBACCO MOSAIC VIRUS IN TURKISH  
TOBACCO (*NICOTIANA TABACUM*)

By

J. P. VARMA and G. S. VERMA

Department of Botany, Lucknow University, Lucknow

[Received on January 13, 1963]

Results on the studies on multiplication of plant viruses in relation to nitrogen nutrition have been contradictory. This may, perhaps, be due to variations in growing medium (washed sand or soil), salt or fertilizer used, host virus combinations and experimental methods. Concentration of tobacco mosaic virus (TMV) was found equal in nitrogen deficient and not deficient tomato plants by Rischkov and Smirnova (1939). Spencer (1939) concluded that nitrogen increased susceptibility of the host (tobacco plant), TMV infectivity and TMV production. Bawden and Kassanis (1950) reported that increased nitrogen (fertilizer) increased host susceptibility and TMV concentration in tobacco as long as the plant size was increased. Weathers and Pound (1945) claimed that TMV in expressed juice was inversely proportional to nitrogen supply, in tobacco plants, although purified TMV was found to be directly correlated.

The present paper reports the effect of nitrogen starvation on height, fresh weight, symptom severity and active tobacco mosaic virus formation in Turkish tobacco (*N. tabacum* L.) in sand culture.

**Material and Methods :**

The methods of sowing, transplanting the host (*N. tabacum* var Turkish) plants, the technique of sand culture used in these studies and the mode of harvesting have been described in earlier papers (Varma and Varma, 1961, 1962). The virus inoculum was the sap obtained by crushing the fresh infected leaves of Turkish tobacco on which TMV was cultured. Inoculations were done with the forefinger dipped in inoculum. The nutrient solution, described by Arnon and Hoagland (1940) was formulated to contain different concentrations (0, 28, 252 and 3052 ppm) of nitrogen. For 0 and 28 ppm,  $\text{KNO}_3$ ,  $\text{Ca}(\text{NO}_3)_2$  and  $\text{NH}_4\text{H}_2\text{PO}_4$  salts were replaced by  $\text{K}_2\text{SO}_4$ ,  $\text{CaCl}_2$  and  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  respectively.

Three separate conditions i.e. (a) No nitrogen starvation (b) Partial nitrogen starvation and (c) Complete nitrogen starvation, were maintained. In each condition 36 uniform seedlings were transplanted 20 days after sowing in 3 batches. In condition (a) i.e. no nitrogen starvation each batch was supplied with 28, 252 and 3052 ppm of nitrogen respectively after transplantation. In condition (b) low dose (28 ppm) of nitrogen was given to every batch of plants for 2 weeks after transplantation. Thereafter 28, 252 and 3052 ppm of nitrogen was restored to each batch respectively. In condition (c) plants were transplanted in 3 batches to nitrogenless (0 ppm) sand. In this condition plants developed severe deficiency in 2 weeks and henceforth 28, 252 and 3052 ppm of nitrogen supply was restored to 3 batches respectively as before.

The plants growing in 3 different conditions of nutrient supply for 3 weeks after transplantation were inoculated with the virus. Control plants were not inoculated. Four weeks after inoculation they were harvested.

At harvest, the average height (cm.) and fresh shoot weight (g.) of inoculated and uninoculated plants were taken. The leaves were removed and kept in deep-freeze. After a day or so they were homogenized with distilled water in the proportion of 1 ml. of distilled water to 1 g. of fresh leaf tissue and the juice expressed through muslin cloth was kept frozen until virus assay. For determination of active virus the juice was first clarified by low speed (3,000 r.p.m.) centrifugation. Aliquots were diluted 1/10 in sterile distilled water and relative virus production was determined by counts of local lesions produced on half leaves of *Nicotiana glutinosa* L. For determination of virus concentration, *N. glutinosa* plants were arranged on the basis of Latin sq. arrangement. Such an arrangement involved 4 blocks of 3 plants each of *N. glutinosa*. Each plant was trimmed to exactly 3 comparable leaves. In such arrangement each of the 3 samples had 24 replications and occurred 6 times in each of the 4 blocks, eight times on each leaf position and twelve times each on the left and on the right half leaf. Average number of local lesions per half leaf were calculated.

### Results :

In normal case the leaves of uninoculated plants grown at 28 ppm of nitrogen concentration became chlorotic. At 3052 ppm of nitrogen concentration they were dark green in the beginning, later developing slight yellowing with necrotic areas on them. TMV infection produced appreciable distorting and crinkling of leaves in these plants.

The height and fresh weight of Turkish tobacco were adversely affected with low (28 ppm) and high (3052 ppm) level supply of nitrogen. Medium (352 ppm) nitrogen was found optimum for growth. Virus infection, however, caused overall loss in height and fresh weight. The percent loss in height and fresh weight due to virus infection was directly proportional to available supply of nitrogen in the nutrient solution. A spell of starvation prior to virus inoculation increased the percent loss in height and fresh weight of the infected plants (Table 1).

Active virus increased with increasing concentration of nitrogen. A spell of nitrogen starvation of the host plant prior to virus infection and supply of 28, 252 and 3052 ppm of nitrogen decreased active virus formation. Complete starvation (0 ppm) decreased the production of active virus more than the partial starvation (28 ppm) in every case (Table 1).

### Discussion :

The investigations, as presented here, reveal that TMV infection decreased the height and fresh shoot weight of Turkish tobacco. The systemic disease symptoms appeared at all levels of nitrogen; a marked deficiency (0 ppm) of nitrogen limited the effects of TMV infection which could be attributed to reduced active TMV production in plants deficient in nitrogen. Normally the percent loss in height and fresh shoot weight of the host plant due to virus infection increased with increasing supply of nitrogen but a spell of nitrogen starvation prior to virus infection further increased percent loss and also disturbed the normal response of the host to different levels (28, 252 and 3052 ppm) of nitrogen supply. This should indicate that nitrogen starvation depressed the normal metabolic processes. Complete nitrogen starvation (0 ppm) affected greatly than partial starvation (28 ppm). The manifestation of height and fresh weight of virus infected plants on account

of starvation-spell before inoculation had no relationship with active virus production.

TABLE 1  
Effect of nitrogen starvation on height, fresh weight and active TMV in Turkish tobacco (*N. tabacum*) plants grown in sand culture

Experiment	Levels of nitrogen restored	Height of shoot (cm.)		% loss in height due to virus infection	Fresh weight of shoot (g.)		% loss in weight due to virus infection	Active virus*
		Healthy	Diseased		Healthy	Diseased		
No nitrogen starvation (normal control)	28 ppm	3.0	2.7	10%	2.5	1.8	28%	25.0
	252 ppm	30.0	24.5	18%	61.0	25.0	59%	70.0
	3052 ppm	8.0	4.0	50%	17.0	6.0	65%	108.0
Partial nitrogen starvation	28 ppm	2.3	1.84	20%	1.9	0.9	53%	27.2
	252 ppm	7.7	4.1	47%	21.0	3.9	81%	46.3
	3052 ppm	8.3	2.7	67.5%	13.8	4.5	67%	80.0
Complete nitrogen starvation	28 ppm	4.7	3.0	36%	3.0	1.85	38%	8.0
	252 ppm	11.7	3.4	71%	31.85	2.75	91%	26.6
	3052 ppm	6.4	3.4	47%	10.75	2.0	81%	61.2

\*Average No. of local lesions per half leaf based on 24 half leaves of *N. glutinosa*.  
Height and fresh weight values are the averages of 6 plants.

Active TMV gradually increased with increasing levels of nitrogen. It is obvious that starvation decreased the active virus production when compared with no starvation (normal). Complete starvation (0 ppm) had more adverse effects on active TMV production than partial starvation (28 ppm). The experimental results as presented here also show that the growth of plants (height and fresh shoot weight) is not necessarily in accord with the active TMV concentration in the sap. This varies with the view and findings of Bawden and Kassanis (1950) with respect to the nitrogen nutrition in relation to multiplication of TMV. This may perhaps be explained on the consideration that Bawden and Kassanis (1950) used soil in their studies and did not use nitrogen levels high enough to cause stunting of plants while experiments presented here were done in sand culture. The findings presented here are also contrary to those of Rischkov and Smirnova (1939) who claimed that TMV concentration in nitrogen deficient tomato plants equalled to those adequately supplied and that of Weathers and Pound (1954) who are of the opinion that TMV concentration in expressed juice is inversely correlated with the increase of nitrogen supplied in host's nutrition. However, these findings agree in some respects with Spencer (1939) who described in his sand culture studies that virus activity of expressed juice was directly correlated with the amount of nitrogen supplied and Chessin (1951) who found that nitrogen deficiency affected some properties of TMV multiplied in tobacco.

Nitrogen is the component part of TMV usually forming 16% of the total weight. Therefore, it appears feasible to say that synthesis of virus is limited by nitrogen starvation of the host plant in which it multiplies. This relationship may be direct or through normal plant proteins, as Wildman and Bounner (1950) have reported that virus protein synthesis represents some, although not complete,

break down of normal proteins followed by a new and constitutionally different synthesis or as pointed out by Bawden and Kassanis (1950) that TMV multiplies at the expense of normal plant proteins. Verma and Varma (1962) have also indicated that active virus is correlated to virus protein which is also related to leaf-sap protein. Nitrogen might increase TMV multiplication indirectly also as pointed by Burries (1959) that plants accumulate organic acids when cultured on nitrate or ammonia. Organic acids at the same time have been found by Schlegel (1957) to stimulate TMV multiplication.

#### Summary :

Increase in the supply of nitrogen to the host plant (*N. tabacum*) in sand culture increased symptom severity, percent loss in its height, fresh shoot weight and active TMV production. A spell of starvation prior to TMV infection, however, increased percent loss in height and fresh shoot weight and decreased active virus formation.

#### Acknowledgments :

The authors are grateful to Dr. B. M. Gupta of Central Drug Research Institute, Lucknow, for help.

#### References :

- Arnon, D. I. and Hoagland, D. R. Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. *Soil Sci.*, **50** : 463, 1940.
- Bawden F. C. and Kassanis, B. Some effects of host plant nutrition on the multiplication of viruses. *Ann. appl. Biol.*, **37** : 215, 1950.
- Burries, R. H. Nitrogen nutrition. *Ann. Rev. Plant physiol.*, **10** : 301, 1959.
- Chessin, M. The effects of nitrogen deficiency on the properties of tobacco mosaic virus. *Phytopathology*, **41** : 235, 1951.
- Rischkov, V. L. and Smirnova, V. A. Accumulation of virus of tobacco mosaic in plants when nitrogen is withheld from them (abstract). *Rev. appl. Mycol.*, **18** : 715, 1939.
- Schlegel, D. E. The stimulatory effect of organic acids on tobacco mosaic virus multiplication. *Virology*, **4** : 135, 1957.
- Spencer, E. L. The effect of host nutrition on concentration of tobacco mosaic virus. *Plant Physiol.*, **14** : 769, 1939.
- Verma, G. S. and Varma, J. P. The effect of potassium nutrition on multiplication of tobacco mosaic virus in Turkish tobacco (*Nicotiana tabacum* L.) plants. *Indian J. Microbiol.*, **1** : 4, 1961.
- Effect of potassium deficiency on tobacco mosaic virus multiplication in Turkish tobacco (*Nicotiana tabacum*). *Acta virol.*, **6** : 181, 1962.
- Weathers, L. G. and Pound, G. S. Host nutrition in relation to multiplication of tobacco mosaic virus in tobacco. *Phytopathology*, **44** : 74, 1954.
- Wildman, S. G. and Bonner, J. Chemical and physical evidence that tobacco mosaic virus is derived from a main nucleoprotein component in tobacco leaf cytoplasm. *Phytopathology*, **40** : 31, 1950.

# STUDIES IN THE TAXONOMY AND CYTOLOGY OF CERTAIN SPECIES OF *ANEILEMA SENSU LATO* IN EASTERN INDIA

By

G. PANIGRAHI and R. V. KAMMATHY

*Botanical Survey of India, Allahabad*

[Received on March 16, 1963]

The genus *Aneilema* R. Br. (1810), represented by about 100 species in the world flora, is distributed in the warmer parts of the world. Hooker (1894), Fischer (1931), and Blatter (1926-1935) and other taxonomists working on Indian flora record 31 species from different parts of India. The genus along with the other genera of the family Commelinaceae have been revised by various workers viz. Hasskarl (1866, 1870), Clarke (1874, 1881), Hooker (1894), Brückner (1926, 1930), Pichon (1946) and others. Brückner (1926) created a new genus, *Phaeneilema* Brückner and transferred many species of *Aneilema sensu lato* to this new genus. Subsequently he (1930) realised that *Phaenelima* Brückner (1926) was antedated by *Murdannia* Royle (1839) and, therefore, transferred many species of *Aneilema* R. Br. and all the species of *Phaenelima* Brückner to *Murdannia* Royle. Brenan (1952) and Raizada (1958) and other recent workers support Brückner's (1930) treatment. The distinctions between the two genera are based on the following characters (cf. Diagram I).

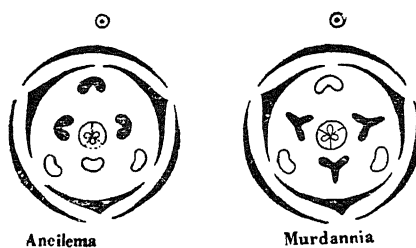


DIAGRAM I

*Aneilema* : 3 anterior stamens (2 antisepalous and one antipetalous) fertile and 3 or 2 posterior stamens (2 antipetalous and 1 antisepalous) represented by bilobed staminode or staminodes or staminodes absent. Capsule usually 2-celled or rarely with an unequal third cell. Cells mostly one seeded, rarely many seeded. The anterior petal usually smaller than the other two.

*Murdannia* : 3 antisepalous stamens fertile (rarely 2), alternating with 3 antipetalous trilobed staminodes. Capsule 3 equal-celled with one to many seeds. Petals almost equal.

Based on the above distinctions 23 out of 32 Indian species have been transferred to *Murdannia* by various workers. Royle (1839) transferred only one species, Brückner (1930), fifteen species, Brenan (1952), three species and Raizada (1958),

four species. *Aneilema protensum* Wall., *A. thomsoni* Clke., *A. conspicuum* Kunth, *A. montanum* Wt. (Text fig. 13) may be treated as the four Indian species, belonging to typical *Aneilema sensu stricto*. The generic affinity of the remaining species absent from Eastern India, viz. *A. hallbergii* Blatter, *A. dimorphum* Dalz., *A. rigidum* Blatter, *A. pulneyense* Fyson and *A. ovalifolium* Hk. f. ex. Clke. await further study of the floral parts in live specimens, whereas *A. aequinoctiale* Kunth (*Amelima wallichii* Clarke from Darjeeling) is treated as an *excluded species* from India.

The assignment of the East Indian species of *Aneilema sensu lato* under *Aneilema* R. Br. *sensu stricto* and *Murdannia* Royle and their arrangement into subgenera and sections as proposed by Clarke (cf. Hooker 1894, p. 366) are presented in Table I. Of the Indian species, only *M. nudiflora*, *M. simplex*, *M. semiteres* (Brenan, 1952) and *M. gigantea* (Hooker 1894, p. 379) extend their westerly range of distribution to Africa.

The recognition of *Aneilema* and *Murdannia* as two distinct genera and gradual transfer of the species of *Aneilema sensu lato* to *Murdannia* raised interesting problems of nomenclature. Transfer of all but one East Indian species of the section **Euaneilema** to *Murdannia* and retention of all but one species of the section **Dictyospermum** in *Aneilema* raised genuine doubt regarding the affinity of species included in different groups as proposed by Clarke (cf. Table I.). Further, the existence of various morphological forms within the polymorphic species *Murdannia* (= *Aneilema*) *nudiflora* including *A. nudiflora* var. *terminalis* Wight and the problem of nomenclature involved in *M. terminalis* vrs. *M. hamiltoniana* (Wall.) Brück., called for serious taxonomic investigation.

TABLE I

[East Indian species of *Aneilema sensu lato* as proposed by Clarke (cf. Hooker, 1894) and their correct nomenclature.]

Subgen. 1. **Tricarpellaria** Clke. capsule 3-celled, 3-valved.

Sect. 1. **Euaneilema** cells of ovary 2-ovuled, seeds 1 seriate in each cell.

\*Cells of ovary 3-many ovuled, of capsule 1-3 or more-seeded. (See also *Aneilema nudiflorum*).

(a) Flowers panicled on a radical or subradical, rarely leafy scape.

*Murdannia scapiflora* (Roxb) Royle.

(b) Flowering stem leafy, flowers corymbose or panicled.

*Aneilema thomsonii* Clke.

*Murdannia divergens* (Clke.) Brück.

*Murdannia hookeri* (Clke.) Brück.

*Murdannia elata* (Vahl) Brück.

*Murdannia spirata* (Linn.) Brück.

(c) Stem leafy. Cymes 1-3 flowered, axillary.

*Murdannia triquetra* (Wall.) Brück.

\*\*Cells of ovary 2-ovuled, of capsule 2-seeded (3-seeded in *Aneilema nudiflorum* var. *compressa*).

*Murdannia nudiflora* (L.) Brenan.

*M. nudiflora* var. *terminalis* (Bl.) Panigr. comb. nov.

*Murdannia simplex* (Vahl) Brenan.

*Murdannia gigantea* (Vahl) Brück.

Sect. II **Dichaespermum**. Cells of ovary 4–20 ovuled. Seeds 2-seriate in each cell.

\*Flowers axillary, solitary or clustered, pedicels joined in the middle.

*Murdannia hamiltoniana* (Wall.) Brück.

Sect. III **Dictyospermum**. Cells of ovary 1-ovuled, of capsule one-seeded or empty.

\*Capsule glabrous.

*Murdannia vaginata* (L.) Brück.

*Aneilema montanum* Wight.

*Aneilema conspicuum* Kunth.

*Aneilema protensum* Wall.

#### Excluded species :

*A. aequinoctiale* Kunth (= *Amelima wallichii* Clke.).

This paper presents our observations on the morphology and habitat of some of the East Indian species of *Aneilema sensu lato*. Correct nomenclature with the citation of literature, notes on important morphological characters of taxonomic value, and on habitat and distribution are appended to each species in Section I. Section II deals with the cytological behaviour of the species investigated up-to-date, whereas some of the main points arising out of these studies are discussed in Section III.

#### SECTION I.

**Murdannia scapiflora** (Roxb.) Royle, *Illustr. Bot. Himal.* 403, t. 95, 1839.  
*Commelina scapiflora* Roxb. *Fl. Ind.* 1 : 175, 1832.

*Aneilema scapiflorum* Wt. Ic. t. 2073, 1853 ; Hook. f. *F. B. I.*, 6 : 375, 1894.

A perennial herb with radical leaves and fasciculated tuberous roots. Leaves 10–20 × 1.5–2 cm., margins crisped. Scape 30–50 cm.; inflorescence terminal with eccentric leafy bracts subtending the cymes. Flowers white or light purple; sepals 3; petals 3; stamens 3, fertile; staminodes 3, each trilobed; filaments of all bearded; capsule 3-celled; seeds straw coloured, 3-angled, 4–6 in each cell. (Text fig. 1).

On the forest floor in sandy soil, growing from moniliform tubers.

Assam : Goalpara (30 m.) *U. N. Kanjilal* 5409.

Orissa : Dandakaranya (60 m.) *Rolla* 18590.

*Distribution* : West and South India, tropical and temperate Himalayas, Ceylon and Bhutan.

**Murdannia divergens** (Clke.) Brück. in *Engl. and Prantl. Nat. Pfl. ed.* 2, 15a : 173, 1930.

*Aneilema divergence* Clke. *Commel. et Cyrt. Beng.* 28 t. 16, 1874 ; Hook. f. *F. B. I.*, 6 : 376, 1874.

A stout erect perennial herb 30–100 cm. tall with stout tuberous roots and with 3–4 tillers arising from the same root stock; both stem and leaves glabrous or slightly hairy. Leaves 10–15 × 2–3 cm., leaves leathery with raised longitudinal veins; leaf sheath 3 cm long, clasping the stem upto 1.5 cm.; hairs present on fusion commissure. Scape leafy; inflorescence terminal and highly branched with

small eccentric persistent bracteoles. No anthocyanin present in stem and leaf. Flowers pink; sepals 3, greenish pink; petals 3, subequal, ovate, shortly clawed, pink in colour; stamens 3, fertile, alternating with 3 trilobed staminodes in the inner whorl; filaments of all pinkish and bearded; anthers dorsifixed, dark chocolate in colour, connective dehiscent longitudinally on either side; style linear; stigma purple; capsule 0.5–1 cm. long, equally 3 celled, each cell with 4–6 seeds; seed greyish brown and slightly rugose. (Text fig. 2.)

On hill slopes between 1300 m. to 1900 m., amidst grasses.

Assam: Khasi Hills (1600 m.) *Gustavemann* 562, 1154; Shillong (1500 m.) *P. C. Kanjilal* 8331; Kohima (1600 m.) *Deka* 16518; Naga Hills (1500 m.) *Bor* 21230, Druphal *Bor* 22214; Nongstoin *Panigrahi* 16527; Shillong *Kam-mathy* 29. Manipur. Imphal (80 m.) *Bor* without number.

Distribution: Tropical and subtropical Himalayas, Sikkim and Burma.

**Murdannia hookeri** (Clke.) Brück. in *Engl. and Prantl. Nat. Pfl. ed. 2*, 15a: 173, 1930.

*Aneilema hookeri* Clke. *Commel. et Cyrt. Beng.* 28 t. 17, 1874; Hook. f. *F. B. I.* 6: 376, 1894.

A herb 30 to 45 cm. long, prostrate and rooting at nodes; roots fibrous; leaves 5–7 cm. long, slightly hairy, base clasping the stem. Scape leafy, panicle terminal, repeatedly branched and spreading. Flowers white; sepals 3; petals 3 subequal, white in colour; stamens 3, trilobed; filaments of all bearded; capsule equally 3-celled, each cell with 3–4 seeds. (Text fig. 3).

On the muddy banks of paddy fields and marshes.

Assam: Khasi Hills, Lawlyngdoh (1800 m.) *Bor* 16514; Mawphlong (1950 m.) *Deka* 11089, 32. Orissa: Bhitarkanika (20 m.) *Panigrahi* 23746.

**Murdannia elata** (Vahl) Brück. in *Engl. and Prantl. Nat. Pfl. ed. 2*, 15a: 173, 1930.

*Commelina elata* Vahl, *Enum.*, 2: 178, 1806.

*Aneilema herbaceum* Roxb., Wall. Cat. 5223, 1828.

*Aneilema lineolatum* (Bl.) Kunth *Enum.*, 4: 69, 1843; Hook. f., *F. B. I.*, 6: 376, 1894.

A straight erect herb with tuberous roots. Leaves 10–20 cm. × 4 cm., all leaves radical until erect flowering branches shoot up; leaves glabrous or slightly hairy, somewhat succulent, margins crisped, sheath 4 cm. long. Inflorescence terminal, leafy panicle spreading; sepals 3; petals 3, white (*cf.* Hook. f. p. 377 reporting blue petals); stamens 3, fertile; staminodes 3, somewhat arrow-shaped and bilobed (not trilobed); filaments of all bearded; capsule 0.5 cm. long, equally 3-celled; cells of capsule 4-seeded, seeds angled, minutely tubercled. (Text fig. 4).

The trilobed staminodes characteristic of *Murdannia* (*cf.* Brenan 1952) are absent in this species.

On humus-covered sandy forest floor along with *Hedychium* and on sandy alluvial soil mixed with pebbles.

Assam: Khasi Hills (1500 m.) *Gustavemann* 118, 330; Lumsophedfyreng *U. N. Kanjilal* 7358; Shillong *P. C. Kanjilal* 9259; Umshaw *Sharma* 12370; Sanfan Range *Deka* 22427; Umshaw *De* 210870; Barapani *Saikia* without no. Motharguri (300 m.) *Rolla* 9951, 9977; Tangla *Nath* 13390; Singri *Panigrahi* 14348; Nangkhaw *Panigrahi* 16221; Theria Forest *Deka* 19609 and *De*



19625 ; Lower Cherapunji *De* 19627 ; Kholahat R. F. *De* 19626. N. E. F. A. Kameng F. D. Bhairabkunda *Panigrahi* 15111 ; Aka Hills *Bor* 19016.

Orissa : Batipathar *Panigrahi* 20715 ; Mahadevjharan *Panigrahi* 20830.

Tripura : Cherilam R. F. *Rolla* 8858 ; Chandrapur R. F. *Rolla* 8965.

**Murdannia spirata** (L.) Brück. in *Engl. and Prantl. Nat. Pfl. ed. 2*, **15a** : 173, 1930.

*Commelina spirata* Linn. Mant. 176, 1767.

*Aneilema spiratum* R. Br. Prodr. 271, 1810 ; Hook. f. F. B. I., **6** : 377, 1894.

A small weedy herb, often profusely branched, branches 8–20 cm. and diffused in habit ; leaves sessile, base clasping the stem, leaf blade with prominent raised parallel veins, anthocyanin confined to leaf sheath and older parts of stem only. Panicle leafy, dichotomously branched and chocolate coloured ; bracts narrower than the leaves. Flowers 0.5 cm. in diam., bluish pink ; sepals 3, greenish brown ; petals 3, pinkish violet, subequal ; stamens 3, anthers dorsifixed ; staminodes 3, whitish and trilobed ; filaments of all bearded at base only ; capsule equally 3-celled, each cell with 4 greyish brown seeds, slightly rugose. (Text fig. 5).

In shady areas amidst grasses in pastures and on muddy banks of paddy fields.

Assam : Cherapunji *Deka* 21982, 19209, Shillong *Kammaty* 25.

Orissa : Padampur *Panigrahi* 20337.

Bihar : Silingi *Panigrahi* 12030.

*Distribution* : Common throughout India ; Malaya and China.

**Murdannia triquetra** (Wall.) Brück. in *Engl. and Prantl. Nat. Pfl. ed. 2*, **15a** : 173, 1930.

*Aneilema triquetrum* Wall. Cat. no. 5220, 1828 ; Hook. f. F. B. I., **6** : 378, 1894.

A herb creeping and rooting at nodes ; leaves 5–8 cm.  $\times$  1–1.5 cm., base broad, clasping the stem. Flowers 2–3 in axillary and terminal cymes, slightly protruding out of leaf sheath ; pedicels jointed, decurved or erect ; flowers purple (not blue *cf.* Hook. f. p. 378) ; sepals 3 green, hairy at the tip on the dorsal side ; petals 3, purple ; stamens 3, fertile ; staminodes 3, arrow shaped (not trilobed as in other species of *Murdannia*) ; filaments of all bearded ; capsule equally 3-celled, cells with 4 yellowish brown slightly rugose seeds. (Text fig. 6).

On marshy banks of ditches and paddy fields.

Assam : Guijan, bank of Dibru river *Panigrahi* 21620. N. E. F. A. Subansiri F. D. Hapoli *Panigrahi* 19840.

*Distribution* : China.

**Murdaunia nudiflora** (L.) Brenan in *Kew Bull.* 1952 : 189, 1952.

*Commelina nudiflora* Linn. Sp. Pl. 41, 1753 ; *pro parte*.

*Aneilema nudiflorum* (L.) Wall. List 182, no. 5224, 1830 ; Hook. f. F. B. I., **6** : 378, 1894.

*non M. nudiflora* (L.) Santapau in *Rec. Bot. Surv. India*, **16** (1) : 325, 1953.

A very variable weedy species 10–20 cm., erect or decumbent and rooting at nodes, plant sometimes profusely branched, each branch terminating with 2–3 flowers ; occasionally in older plants, panicle becomes shorter with flowers aggregated at the tip ; sometimes the sessile flowers are sparse or aggregated on one

side of the peduncle. Stem often provided with anthocyanin pigments, leaf sheath and lamina being pubescent. Flowers protected in small oval bracts containing mucilage; flowers blue or bluish pink; sepals 3, green; petals 3, blue, subequal; stamens 2, fertile with bilobed anthers (*cf.* Hook. f. p. 379 reporting 3 fertile stamens), the third stamen being presented by a hairy filament ending in a stump; staminodes 3, trilobed with naked filaments whereas the filaments of the fertile stamens are bearded; capsule equally 3-celled, each cell with 2 brown slightly rugose seeds. (Text fig. 7).

Very common amidst grasses on hill slopes, in moist clayey soil along marshy places along the streams.

*Assam* : Shillong *Kanjilal* 7242 ; Haltugaon *De* 21125 ; Upper Dihing *Panigrahi* 18779 ; Kaziranga *Rolla* 9780 ; Mawphlong *Deka* 19029, 19087 ; Shillong *Kammathy* 26. N. E. F. A. Subansiri F. D. Kimin *Panigrahi* 19427 ; 9th mile along road from Kimin to Ziro *Panigrahi* 19462 ; Tirap F. D. Jairampur *Rolla* 19980 ; Nampong *Rolla* 20081 ; Chenglang *Rolla* 20255 ; Siang F. D. Tuting *Rolla* 17316 ; Pashighat *Deka* 13233.

*Orissa* : Borasambar *Panigrahi* 20647 ; Rebna *Panigrahi* 8448. *Tripuro* : Cherilam R. F. *Rolla* 8866 ; Agartala (Bishalgarh road) *Rolla* 8826.

*Distribution* : Throughout India, N. W. Himalayas upto 1800 m. Burma, Deccan, Travancore, Ceylon.

***Murdannia nudiflora* var. *terminalis* (Bl.) Panigr. comb. nov.**

*Tradescantia termianlis* Bl. *Enum. Pl. Jav.*, i : 6, 1828.

*Aneilema nudiflora* var. *terminalis* (Wt.) *Clke. Mongr.*, 1881 : Hooke f. F. B. I., 6 : 379, 1894.

A stout semiprostrate herb with radical leaves and with branches more or less erect, much less diffused than in *M. simplex*. Inflorescence terminal and also axillary; flowers blue; 2 stamens fertile with the third one represented by a filament; staminodes 3, trilobed; capsule 3-celled, each cell 2-seeded. (Text fig. 7a).

On humus-covered surface of rock; where water trickles down big clones are formed at times; also on sandy alluvium.

*Assam* : Dawki *Deka* 19674, Cherapunji *Deka* 22261 ; Upper Dihing *Kammathy* 21 ; Lekhapani *Panigrahi* 18997. N. E. F. A. : Aka Hills *Bor* 15199 ; New Head Quarters *Panigrahi* 19401 ; Tirap F. D., Laju *Panigrahi* 14730 ; Jairampur *Rolla* 19972.

***Murdannia simplex* (Vahl) Brenan, *Kew Bull.* 1952 : 186, 1952.**

*Commelina simplex* Vahl, *Enum.*, 2 : 177, 1806.

*Aneilema sinicum* "(Sinica)" Ker-Gawl. in *Bot. Reg. t.* 659, 1822 ; Hook f. F. B. I., 6 : 379, 1894.

A perennial herb with radical leaves and tuberous roots; 7-8 branches (tillers) arise from the same root-stock; each branch 30-50 cm. long and diffused, spreading on all sides, tips erect; stem and leaf sheath hairy and provided with abundant anthocyanin and is brown-red with numerous conspicuous needle-like white hairs. Inflorescence once dichotomously branched. Flowers protected by small bracts and contain abundant mucilage. Old peduncles scarred with flowers and fruits with helicoid arrangement. Flowers 3 cm. across; sepals 3, free, green; petals 3, bluish violet (*cf.* Hook. f. p. 374 reporting blue petals); stamens 2, fertile, the third stamen represented by a filament only; filaments bearded; staminodes 3,

trilobed and with naked filaments; capsule 3-celled, each cell with two brown slightly rugose seeds. (Text fig. 8).

Common on the hill slopes with grasses, on humus-covered rocks; sandy alluvial soil in wet places and on road sides. It is a new record for Eastern India.

*Assam*: Theria *Deka* 19603; Sylhet *Purkayastha* 12308; Nongstoin *Panigrahi* 16291; Jowai *Rolla* 2537; Reliang *Rolla* 2636; Shillong *Kammathy* 27, 65, 69.

*Distribution*: Deccan, Ceylon, China and Malaya.

***Murdannia gigantea*** (Vahl) Brück. in *Eng. and Prantl. Nat. Pfl. ed.* 2, 15a: 173, 1930.

*Commelina giganteum* Vahl, *Enum.*, ii: 177, 1806.

*Aneilema giganteum* Br., *Prodr.* 271, 1810; Hook. f. *F. B. I.*, 6: 379, 1894.

A tall erect herb with slender tuberous roots (*cf.* Hook. f. p. 379 reporting fibrous roots), about ten erect tillers arising from the same root stock, stem with long internodes, glabrous and without anthocyanins; leaves long grass-like, 15–25 cm. × 1 cm., anthocyanin confined to leaf sheath only. Inflorescence terminal with one dichotomy and scars of old flowers seen on old peduncles. Flower buds protected in small oval bracts containing abundant mucilage, flowers 2 cm. across; sepals 3, green; petals 3, bluish violet, subequal; only 2 stamens fertile (not 3, as reported by Hook. f. p. 379) and only a bearded filament representing the third fertile stamen; staminodes 3, trilobed and yellow alternating with the fertile stamens; filaments of fertile stamens bearded in the middle only, those of staminodes naked; capsule 3-celled, each cell with two compressed dark brown slightly rugose seeds; some capsule contain only 5 seeds. (Text fig. 9).

Common along moist drains and on grassy hill slopes along with *M. simplex*.

*Assam*: Cherrapunji *Eor* 16516; Laillyngkot *De* 16917; Pongtung *Deka* 22418; Pynursla *Panigrahi* 3093; Shillong *Panigrahi* 3312; Shillong *Kammathy* 24.

*Distribution*: Deccan, Ceylon, Nicobar Malaya, China, Australia, Africa.

***Murdannia blumei*** (Hassk.) Bakhuizen fil. (*see* Raizada *Indian For.* 84: 499, 1958).

*Dichaespermum blumei* Hassk. *Commel. Ind.*, 41, 1870.

*Murdannia hamiltoniana* (Wall. ex Clke. Brück.) in *Engl. and Prantl. Nat. Pfl. ed.* 2, 15a: 173, 1930.

*Aneilema hamiltonianum* Wall. Cat. no. 5222, 1828 *nomen nudum*; Hook. f. *F. B. I.*, 6: 380, 1894, Clarke in *Commel. et Cyrt. Beng.* 1874; *Dc. Monogr.* 1881.

*Murdannia terminalis* (Bl.) Raizada *Indian For.*, 1958.

*M. blumei* (Hassk.) *Rolla Proc. Indian Sci. Cong.*, 1958.

Perennial herb 30–40 cm., semi-erect and rooting at nodes; leaves 5–8 cm. × 1.5 to 2 cm. Flowers white; sepals 3; petals 3; stamens 3, filaments naked; staminodes absent; capsule equally 3-celled; seeds small, black, 15–20 per cell in 2 rows. (Text fig. 10).

This species grows along marshes and swamps, along banks of rivers and road sides.

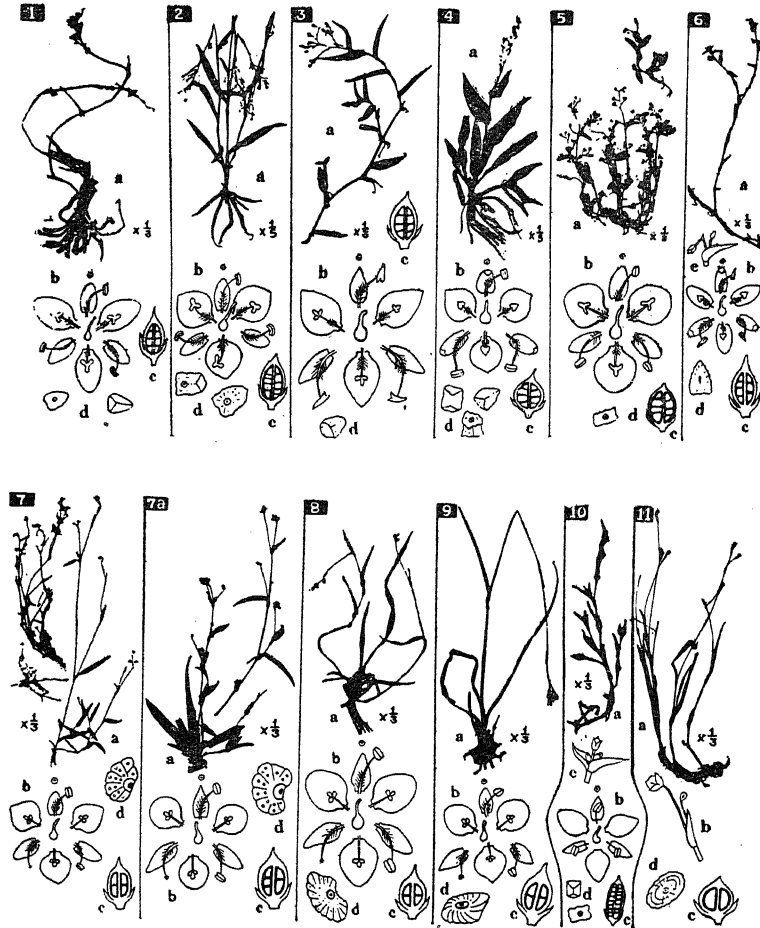
*Assam*: Sonapur (Barnihat) *Panigrahi* 4476; Guijan, bank of Dibru river *Panigrahi* 21621.

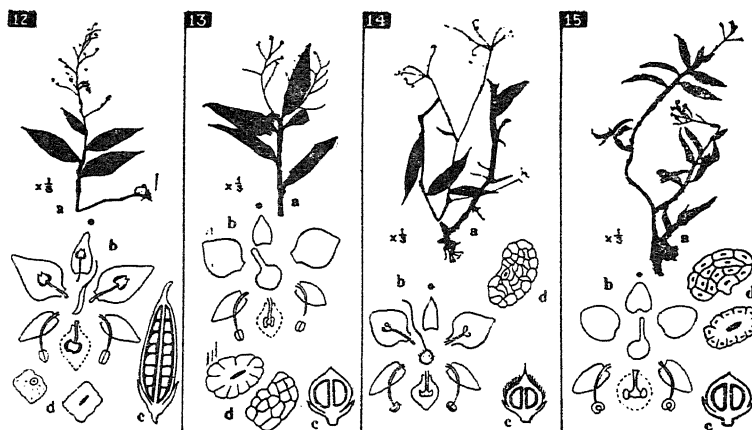
*Distribution*: Upper Gangetic plains to Assam, Java.

# LEGEND FOR TEXT FIGS. 1-15

a—depicts the habit of the species ; b—the arrangement and nature of floral parts ; c—the l. s. of the capsule showing the numbers of seeds per row in one cell ; d—the shape, size and external features of seed ; e—mode of insertion of flowers on the pedicels. (magnification of text figures represented by 'a' is indicated against each ; for those 'b-e', magnification is proportional to that of 'a'.)

1. *Murdannia scapiflora*, 2. *M. divergens* (n=30), 3. *M. hookeri*, 4. *M. elata* (n=21), 5. *M. spirata* (n=20), (2 plants to show ranges of variation), 6. *M. triquetra* (2n=40), 7. *M. nudiflora* n=1'; 3 plants showing range of variation); 7a. *Murdannia nudiflora* var. *terminalis* (2n=39) 8. *M. simplex* (n=40) 9. *M. gigantea* (n=11) 10. *M. blumei* 11. *M. vaginata* (2n=40). 12. *Aneilema thomsonii*, 13. *A. montanum* (n=14), 14. *A. protensum* (n=59), 15. *A. aequinoctiale* (n=24).





Both Haines (1924) and Raizada (1958) cite *Aneilema hamiltonianum* Wall. ex Clke. as synonymous to *A. terminalis* (Bl.) Haines and *Murdannia terminalis* (Bl.) Raizada, respectively, based on *Tradescantia terminalis* (Bl.) Haines (l. c. p. 1080) remarks that oldest specific name for typical *A. hamiltonianum* Wall. Cat. no. 5222 from Assam is *A. terminalis* (Bl.) but this was subsequently reduced by Clarke to a variety of *A. nudiflorum*. But Blume (1828) described *Tradescantia terminalis* Bl. as having the flowers solitary and terminal and growing on grassy plains while *A. hamiltonianum* Wall. ex Clke. has axillary pedicellate flowers and is never found in grassy plains but in marshy areas, ditches and other wet places. It is, therefore, incorrect to treat *terminalis* Bl. and *hamiltonianum* Wall. as conspecific and so are Haine's and Raizada's citations of *A. hamiltonianum* Wall. as synonymous to *M. terminalis* (Bl.) Raizada. Considering *T. terminalis* Bl. (whose type sheet cannot be traced either at Rijksherbarium, Leiden or at Bogor), as a species of *Cyanotis* but not of *Aneilema*, Rolla (1958) disagrees with Raizada (1958) regarding conspecific nature of *A. terminalis* (Bl.) and *A. hamiltonianum* Wall., cites *A. hamiltonianum* Wall. ex Clke. as synonymous to *Murdannia blumei* (Hassk.) Rolla. While we maintain that *M. terminalis* (Bl.) Raizada and *M. hamiltoniana* (Wall.) Brück. cannot be conspecific, *M. blumei* (Hassk.) Bakhuizen fil. antedates *M. blumei* (Hassk.) Rolla and therefore, is adopted here, since *A. hamiltonianum* Wall. (1828) is a *nomen nudum* and Hasskarl (1870) had described the species under *Dichaespermum blumei* Hassk. prior to Clarke (1874, 1881) validating the name *hamiltonianum* Wall. by furnishing the description.

***Murdannia vaginata* (L.) Brück. in Engl. and Prantl. Nat. Pfl. ed., 2 15a : 137, 1930.**

*Commelina vaginata* L., Mant. 177, 1767.

*Aneilema vaginatum* R. Br. Prodr. 271 1810 ; Hook. f. F. B. I., 6 : 381, 1894.

A small slender erect herb of 30-50 cm. profusely branched. Leaves 8-10 cm.  $\times$  0.5-1 cm. Flowers 1-3, axillary and terminal, subtended by an erect convolute bract, about 1 cm. long. Pedicel twice jointed ; capsule globose shining, equally 3-celled, each cell with a single large whitish seed with radiating lines. (Text fig. 11).

On the banks of rice fields and marshes.

Assam : Bholaganja (Sylhet) *Deka* 12480 ; Belsiri river bank (Orang. R. F) *Panigrahi* 14268.

Orissa : Borasambar *Panigrahi* 20647. (It is a new record for Orissa).

**Distribution :** India, Bengal, Deccan, Ceylon, China.

**Aneilema proteusum** Wall. Cat. no. 5218, 1828.

*A. scaberrimum* b. f. Enum. IV : 69, 1843 ; Hook. f. F. B. I., 6 : 382, 1894.

A stout erect herb, 1-2 m. in height ; leaf surface coarse, hairy, leaves 10-15 cm., leaf sheath  $2\frac{1}{2}$ -3 $\frac{1}{2}$  cm., hairs often persistent. Inflorescence terminal and in axillary panicles with branches radiating ; flowers white or whitish blue ; sepals 3 ; petals 3, very delicate, 2 long-clawed, anterior one smaller ; stamens 3 fertile, nearer to the smaller petal ; staminodes 2-3, bilobed ; filaments of all naked ; capsule 3-celled, one cell slightly smaller containing one greyish brown slightly rugose seed per cell. (Text fig. 14).

On the banks of ditches and streams, in shady areas on hill slopes and trails through bushes and grasses, used as fodder for buffaloes.

Assam : Khasi Hills *Gustavemann* 353 ; Nongstoin *U. N. Kanjilal* 5969 ; 6th. mile along Shillong-Gauhati Road *P. C. Kanjilal* 8678 ; Pongtung *De* 19624 ; Naga Hills *Bor* 21354 ; Jowai *Panigrahi* 4116 ; Kakoi R. F. *Panigrahi* 11371. N. E. F. A. : Subansiri F. D. *Kimin Panigrahi* 19758 ; Lohit F. D. Sonogodam (1050 m.) *Rolla* 10260, 10318 ; Paya (300 m.) *Rolla* 10626 ; Parasuram kunda (200 m.) *Rolla* 10929 ; Siang F. D. *Koppu Rolla* 17454 ; Kerim forest (Sadiya) *Deka* 16933 ; Sadiya *Deka* 12704 ; Tirap F. D. *Jairampur Rolla* 19998.

**Aneilema thomsonii** Clke. Journ. Linn. Bot. Soc., XV : 121 1877 ; Hook. f. F. B. I., 6 : 376, 1894.

A stout erect herb, 40-50 cm. tall ; leaves 5-12×3.6 cm. oblong, hairy. Panicle terminal, spreading : sepals 3 ; petals 3 ; stamens 2 fertile on the anterior-side, one antepetalous stamen on the anterior side is peculiar with 2 sac-like structures on a filament ; staminodes 3, bilobed on the posterior side ; filaments of all naked ; capsule 3-celled, 1 slightly smaller, each cell 6-8 seeded, seeds big angular with slightly raised radiating lines. *Brenan* (1952) also observed bilobed staminodes and other characters typical of *Aneilema* in *A. thomsonii* and *A. protensum*. (Text fig. 12).

Assam : Kohima (2100 m.) *Bar* 15808 ; Naga Hills *Bor* 21353 ; N. E. F. A. : Aka Hills *Bor* 22119 ; Kameng F. D. *Rolla* 1248 ; Lohit F. D. *Dreyi* (1200 m.) *Rolla* 10503 ; Shoenliang (625 m.) *Rolla* 10525 ; Heyuliang (491 m.) *Rolla* 10724.

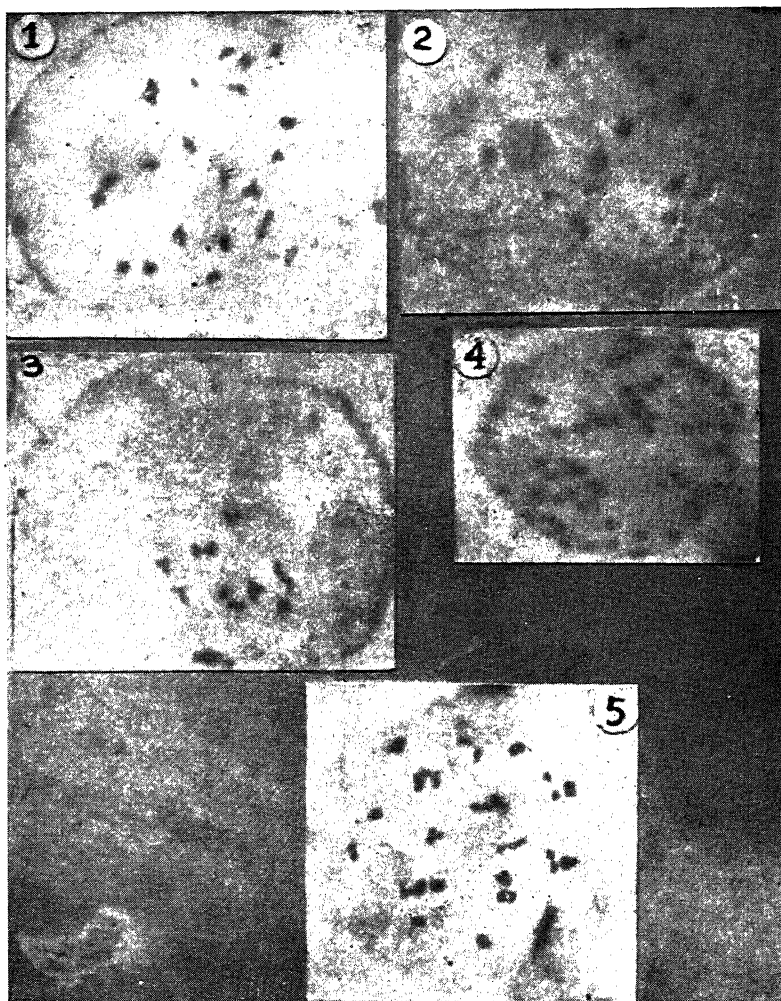
*Distribution* : Eastern Himalayas, Sikkim and Bhutan.

**Aneilema aequinoctiale** Kunth, Enum. IV : 72, 1843 ; Clarke Monogr. 221.

*Amelima wallichii* Clke. Commel. et Cyrt. Beng. 38 t. 26, 1874.

A tufted stout herb 30 cm. Leaves ovate, lanceolate, glabrous, leaf sheath persistent, hairy at the tip. Inflorescence terminal, cymes 3-4 flowered ; flowers light purple ; sepals 3, greenish, posterior one slightly smaller ; petals 3, anterior one slightly smaller ; stamens 3, fertile, on the anterior side ; filaments naked, staminodes absent. (Text fig. 15).

West Bengal : Darjeeling *Panigrahi* 20023.



LEGEND FOR PLATE 1. (All Microphotographs X 900).

Plate I. 1. *Murdannia elata*—One cell at diakinesis with  $n=21$ .

2. and 3. *M. nudiflora*—One cell at diakinesis showing  $n=10$  and another cell with 10 chromosomes in one young pollen grain.

4. *Aneilema protensum*—One cell at diakinesis with 59 bivalents.

5. *A. aequinoctiale*—On cell at diakinesis with 22 bivalents and one tetravalent.

## SECTION II

### Cytology :

#### **Murdannia divergens** (Clke.) Brück.

Shillong biotypes show  $n=30$ . Although it is difficult to discuss the configuration of the bivalents at diakinesis, owing to their greatly contracted nature, two bivalents appear to be loosely associated; of the three bivalents seen attached to the large nucleolus placed on one side of the cell, two pairs are somewhat rod-shaped. Analysis of 5 clear cells do not show any univalents. (Panigrahi and Kammathy 1963).

#### **Murdannia elata** (Vahl) Brück.

The biotypes from Barnihat, Assam turn up with  $n=21$  (Plate I, fig. I) and with  $2n=42$  in root tips. The bivalents are, however, small with 4 pairs x-shaped, 2 pairs rod-like; 6 pairs V-shaped, and the remaining dot-like. Sharma and Sharma (1958), however, report  $2n=40$  in *Aneilema herbaceum* Wall. from Rongo (4000'-5000') in West Bengal.

#### **Murdannia spirata** (L.) Brück.

Biotypes from Shillong show  $n=20$  at diakinesis; most of the bivalents are ring-shaped or x-shaped and one bivalent is observed with loose attachment. There is a large nucleolus on one side of the cell. Sharma and Sharma (1958) reported  $2n=20$  and Murthy (1934) reported  $2n=40$  in the biotypes collected from Darjeeling at 6000' and from plains of South India, respectively. Thus, both diploids and tetraploids occur in this species. But Raghavan and Seshagiri Rao (1961) report  $n=9$  in the biotypes from Mysore, which, therefore, shows the occurrence of a different base number in this species (see also Panigrahi and Kammathy 1963).

#### **Murdannia triquetra** (Wall.) Brück.

Analysis of the root tips of biotypes from Dibru river bank in Upper Assam shows  $2n=40$ .

#### **Murdannia nudiflora** (L.) Brenan.

Shillong biotypes reveal  $n=10$  at diakinesis with a large nucleolus placed on one side of the cell (Plate I fig. 2 & 3). The configuration of the bivalents are difficult to determine owing to their greatly contracted nature although presence of two rod-shaped pairs is evident. Microspores at the tetrad stage show 10 chromosomes, of which 5 are almost metacentric, 1 rod-shaped and 4 others are greatly contracted, almost to dots. This finding is in conformity with the report of  $2n=20$  by Simmonds (1954) from South-East Asia and by Sharma (1955) from plains of West Bengal. No interbivalent connections are, however, observed in any of our cells (cf. Sharma l.c.).

#### **Murdannia nudiflora** var. **terminalis** (Bl.) Panigr.

Cytology of the biotypes from Margherita in Upper Assam shows 20 chromosomes at one pole and 19 chromosomes at another at anaphase I. Another cell at diakinesis possesses 1 trivalent, and 18 bivalents, of which 8 are almost dot-like simulating univalents. Thus, the variety may be characterised by aneuploidy with  $2n=39$ . (cf. Panigrahi and Kammathy 1963).

#### **Murdannia simplex** (Vahl) Brenan.

Cytological analysis of the biotypes occurring in Shillong hills shows  $n=40$  at diakinesis of which 15 bivalents are comparatively much the larger in size in



contrast to the remaining bivalents represented almost as dots, simulating univalents, (cf. Panigrahi and Kammathy 1963).

This count is based upon the analysis of 10 good cells. The plants set good seeds rather profusely and there is no evidence of meiotic irregularity. Considering the report of  $n=20$  in *Aneilema sinicum* in Southern India (Shetty and Subramanyam 1961), the biotypes in Mysore with  $n=30$  (cf. Raghavan and Seshagiri Rao 1961) and in Khasi and Jaintia Hills with  $n=40$  are hexaploids and octoploids respectively, based on  $x=10$  (cf. Panigrahi and Kammathy 1963).

#### **Murdannia gigantea** (Vahl) Brück.

Biotypes growing wild in Shillong possess  $n=11$  at diakinesis with a large nucleolus. 10 of the bivalents are greatly contracted with ring and x-shaped configurations whereas the 11th. one is a rod shaped bivalent. This finding is in conformity with our earlier observations.

#### **Aneilema protensum** Wall.

Cytological analysis of the species from near Barapani near Shillong reveals 59 bivalents at diakinesis, clearly spaced out from one another. (Plate I fig 4). This count is based on not less than 10 different cells. It is of interest to note that the sizes of the bivalents at diakinesis in this species appear much larger than the largest bivalents in *Murdannia simplex*.

#### **Aneilema aequinoctiale** Kunth.

Cytological studies in one Darjeeling biotype show  $n=24$  in one cell, and again, 22 bivalents and one quadrivalent in another cell (Plate I fig. 5).

Thus, it appears that the haploid number in *A. aequinoctiale* is  $n=24$ . However, the bivalents are invariably loosely associated, 4-5 pairs being drawn out with end to end pairing. Not only, therefore, *A. aequinoctiale* provides a different base number viz.  $x=24$ , but shows a completely different karyotypic picture from the remaining species of *Aneilema sensu lato* described above (See also Panigrahi and Kammathy 1963).

### SECTION III

#### **Discussion :**

(a) *Taxonomic* : Study of Table I and other relevant literature suggests that the basis of classification in the genus, *Aneilema sensu lato* is provided by the number of ovules and seeds in each cell of the tricarpellary ovary, all the Indian species of the genus being included in the sub-genus TRICARPELLARIA Clke. While the three sections of the sub-genus are distinguished from each other again on the number of ovules per cell of ovary, (2 ovules in EUANEILEMA, 4-20 ovules per cell in DICHAESPERMUM and one ovule per cell in DICTYOSPERMUM) further sub-division within each section is based on the manner of disposition of the leaves on the flowering axis. Recent authors, however, recognise two genera, viz. *Aneilema* R. Br. and *Murdannia* Royle within the limits of *Aneilema sensu lato* on the manner of disposition of fertile stamens and sterile staminodes with respect to petals and in relation to anterior or posterior side of the mother axis, (Diagram I).

Of the 9 species of the Euaneilema section studied here, *M. scapiflora* with its radical leaves and with the panicle borne on leafless scape and *M. triquetra* with 1-3 flowered, axillary cymes borne on semi-prostrate leafy stems, are easily distinguished from the 7 species in this section.

The four species, *M. divergens*, *M. elata*, *M. hookeri* and *M. spirata* all share panicled inflorescence borne on leafy stems, and 3 fertile stamens alternating with 3 sterile, trilobed staminodes (cf. *M. elata* having bilobed staminodes like those in typical *Aneilema*). Yet, *M. divergens* and *M. elata* on the one hand and *M. hookeri* and *M. spirata* on the other, appear more closely allied to each other, both in general habit and in preference for ecological habitats.

Although *M. nudiflora* (including var. *terminalis*), *M. simplex* and *M. gigantea* share 2-ovuled ovary and 2-seeded capsule with each other, there are rather striking differences between them in their general habit. While *M. simplex* and *M. gigantea* each show great uniformity in morphological features and restricted nature of their distribution in Khasi and Jaintia Hills, *M. nudiflora* is rather a polymorphic species showing a great range of morphological variants spread almost over whole of India in varying ecological niches. *M. nudiflora* var. *terminalis* possesses intermediate morphological features between *M. nudiflora* complex and *M. simplex*.

*M. nudiflora* grows together with *M. spirata* in the Khasi Hills. *M. spirata* with 3 fertile stamens and 3 staminodes may be distinguished from *M. nudiflora* with 2 fertile stamens, a staminal stump and 3 staminodes, but otherwise would need a trained eye to distinguish them from each other in the field. It may, therefore, be reasonably assumed that free interbreeding between the two species might be going on in nature to produce a hybrid swarm, which might include in them the great range of morphological variants within *M. nudiflora* complex.

*M. gigantea* with its striking morphological features can at once be set apart from all other species of *Murdannia*. Similarly, *M. elata* with its bilobed staminodes characteristic of *Aneilema sensu lato* shares its arrangement of stamens and staminodes in the flowers with that of *Murdannia* and, therefore, may serve as a connecting link between the two genera.

*A. aequinoctiale* Kunth from Africa to which *Amelima wallichii* Clke. from Darjeeling is synonymous, is treated as an excluded species from the scope of *Aneilema sensu lato* by Hooker f. (1894). The root stock producing 2 or 3 stout branches at the ground level, the hairy stems and pubescent leaves, older basal stems bearing dried up leaf sheaths, the inconspicuous terminal inflorescence bearing only a few flowers and a few seeds and the Indian distribution of the species restricted to Darjeeling only, mark out the species as very distinctive amongst the Indian species of *Aneilema sensu lato*.

(b) *Cytological* : All available cytological data, including our own, on the genus *Aneilema sensu lato* have been presented in a tabular form and the significance of the discovery of euploidy, aneuploidy and aneuploid base numbers together with the cytogenetical evolution at work within the genus *Aneilema sensu lato* have been discussed in another communication (cf. Panigrahi and Kammathy 1963). It is, therefore, not proposed to discuss these aspects of the study again here.

### Summary :

1. The paper presents our observations on the nomenclature, habit, habitat, distribution and cytology of 11 species of *Murdannia* and a few species of *Aneilema* collected from Eastern India.

2. Nomenclatural problems involving *Murdannia hamiltoniana* (Bl.) Brück, *M. terminalis* (Bl.) Raizada and *M. blumei* (Hassk.) Rolla have been discussed and a new varietal combination *M. nudiflora* var. *terminalis* (Bl.) Panigr. has been proposed for *Aneilema terminale* (Bl.) Wt. and *A. nudiflora* var. *terminalis* (Bl.) Clke.

### Acknowledgement :

Grateful thanks are due to the authorities of the Council of Scientific and Industrial Research, New Delhi for the award of financial assistance including the grant of a Junior Research Fellowship to the Junior author.

### References :

- Blatter, E. Revision of the Flora of Bombay Presidency. In *J. Bombay nat. Hist. Soc.* parts Nos. 3-11, 15-19, 21, 27, 1926-1935.
- Blume, C. L. *Enum. Pl. Jav.*, i : 6, 1828.
- Brenan, J. P. M. Notes on African Commelinaceae. *Kew Bull.*, No. 2. 179-208, 1952.
- Brown, R. *Prodr.*, 271, 1810.
- Brückner, G. *Engl. Bot. Jahrb.*, 61 : *Beibl.*, 137 : 1-70, 1926.
- In *Engl. et Prantl. Nat. Pflanzenf. ed. 2*, 15a : (account on *Murdannia* on p. 173), 1930.
- Clarke, C. B. *Commelinaceae et Cyrtandraceae Bengalenses (paucis allis ex terris adjacentibus additis)*, Calcutta, 1874.
- Notes on Commelinaceae. *J. Bot.*, 19 : 193-202, 1881.
- Commelinaceae in *DC. Monogr. Phan.*, 3 : 1881.
- Fischer, C. E. C. *Flora of the Presidency of Madras*. London. Part 9 : 1533-1552, 1931.
- Haines, H. H. *The Botany of Bihar and Orissa*. London. 6 : 1074-1082, 1924.
- Hasskarl, J. K. In *Flora*, 49 : 212, 1866.
- *Commelin. Ind.*, 41, 1870.
- Hooker, J. D. et al. *The Flora of British India*. London, 6 : 1894.
- Murthy, K. L. Gametogenesis and Embryogeny in some Commelinaceae. *Curr. Sci.*, 3 : 258-259, 1934.
- Panigrahi, G. and Kammathy, R. V. Studies in the Cytomorphology of *Aneilema sensu lato* in E. India. *Proc. 48th Indian Sci. Congr.*, Part IV : 97-98, 1961.
- Cytogenetical evolution in the genus *Aneilema sensu lato* in Eastern India *Memoir 4, J. Ind. Bot. Soc.*, 1963. In press.
- Pichon, M. In *Not. Syst.*, 12 : 217-242, 1946.
- Raghavan, R. S. and Seshagiri Rao, R. *Curr. Sci.*, 30 (8) : 310-311, 1961.
- Raizada, M. B. Name changes in common Indian plants. *Indian For.* p. 499, 1958.
- Royle, J. F. In *Illustr. Bot. Himal.*, 403, t. 95, fig. 3, 1839.
- Seshagiri Rao, R. A preliminary note on the revision of Indian Commelinaceae. *Proc. 45th Indian Sci. Congr.*, Part III : Abstracts p. 331, 1958.
- Shetty, B. V. and Subramanyam, K. Cytology of some taxa of Commelinaceae. *Proc. Indian Sci. Cong. Part III* : Abstracts p. 293, 1961.
- Sharma, A. K. Cytology of some of the members of Commelinaceae and its bearing on the interpretation of Phylogeny. *Genetica*, 27 : 323-393, 1955.
- and Sharma, Archana. Further investigations on cytology of members of Commelinaceae with special reference to the role of polyploidy and the origin of ecotypes. *J. Genet.*, 66 (1) : 1-22, 1958.
- Simonds, N. W. Chromosome behaviour in some tropical plants. *Heredity* 8 : 139-146, 1954.

BIOMETRICAL GROUPING IN *PROTOMYCES MACROSPORUS* UNG.  
CAUSING STEM-GALL DISEASE OF CORIANDER

By

J. S. GUPTA and S. SINHA

Botany Department, Agra College, Agra

[Received on October 23, 1962]

Popta (1889) and Büren (1922) pointed out the possibility of physiologic specialization in *Protomyces*. Some workers have contended that distinct biological forms differ in their comparative morphology of spore forms as shown by Levine (1923, 1928) for *Puccinia graminis tritici* in the characteristics of uredospores. Waterhouse (1930) also suggested that any two biological forms might differ greatly in their spore measurements. Recently, Dalela and Sinha (1957) pointed out the possibility of existence of three biologic forms in *Puccinia penniseti* Zimm. Similar investigations have been conducted here to study variations, if any, among the collections of the parasite (*Protomyces macrosporus*) obtained from different localities. These variants may pave the way for the investigations on physiologic specialization. The diameter of chlamydospores, the thickness of their episporium and the diameter of spore sacs (vesicles) on germination were measured in samples of the fungus obtained from eight widely separated localities.

To eliminate variations existing in the samples of the parasite of each locality due to host varieties and due to environmental conditions, all the samples were raised on a single variety of coriander at Agra and the chlamydospores from the galls developed on this variety were employed in the biometrical analysis.

The infected fruits harvested in the month of April were stored separately in glass-stoppered-bottles at room temperature and observations on germinated spore-sacs were made after a period of dormancy of five months. The chlamydospores were germinated at 20°C.

On a suggestion by Dr. C. R. Rao of the Indian Statistical Institute, Calcutta (private communication), that 'analysis of variance' is a better criterion than the method of frequency curve, the former was adopted for determining the biologic forms on biometrical basis. The results were analysed statistically following the simple 'analysis of variance' method and 'critical differences' (C.D.) were calculated at 5% probability, wherever the results were significant.

**Results :**

Observations on the three characters mentioned above were made and the mean values of all the observations along with their 'critical differences' (C.D.) at 5% level are given in Table I.

TABLE I

Diameter of chlamydo-spores, thickness of episporia and diameter of germinated spore-sacs of *Protomyces macrosporus* Ung. in samples from widely separated localities

No.	Localities	Diameter of Chlamydo-spores (Mean of 100)	Thickness of episporia (Mean of 100)	Diameter of germinated spore-sacs (Mean of 50)
1.	Behraich	47.32 $\mu$	4.46 $\mu$	68.44 $\mu$
2.	Varanasi	52.60 „	4.82 „	69.76 „
3.	Dehradun	52.27 „	5.28 „	64.15 „
4.	Deoria	53.23 „	5.05 „	63.56 „
5.	Jaunpur	49.27 „	4.62 „	63.69 „
6.	Meerut	52.47 „	4.98 „	60.92 „
7.	Moradabad	50.72 „	5.58 „	67.52 „
8.	Patna	49.34 „	5.05 „	65.21 „
C.D.*		1.95	0.43	2.74

\*Critical differences at 5% level.

#### Conclusions :

Diameter of chlamydo-spores :	4	2	6	3	7	8	5	1
Thickness of episporia :	7	3	8	4	6	2	5	1
Diameter of germinated spore-sacs :	2	1	7	8	3	5	4	6

Note : Numbers denote different localities (Table I).

The data reveal that excluding the intermerging samples, two groups are noticed on the basis of diameter of chlamydo-spores :

- (i) Deoria, Varanasi, Meerut and Dehradun, and
- (ii) Patna, Jaunpur and Behraich.

Studying the data on thickness of episporia the differences between the samples from various localities are not very clear. However, Moradabad and Dehradun on the one hand and Jaunpur and Behraich on the other fall into two distinct categories. The rest intermerge with either of the categories. Again, in the case of diameter of spore-sacs two distinct groups are observed, one comprising of Deoria and Meerut ; the other of Varanasi, Behraich and Moradabad while the remaining ones are intermerging.

It is evident from the results that quantitative morphological differences do exist between the samples collected from localities widely separated. On subjection of the data to statistical analysis two distinct categories are noticed but only with regard to any one type of the character studied.

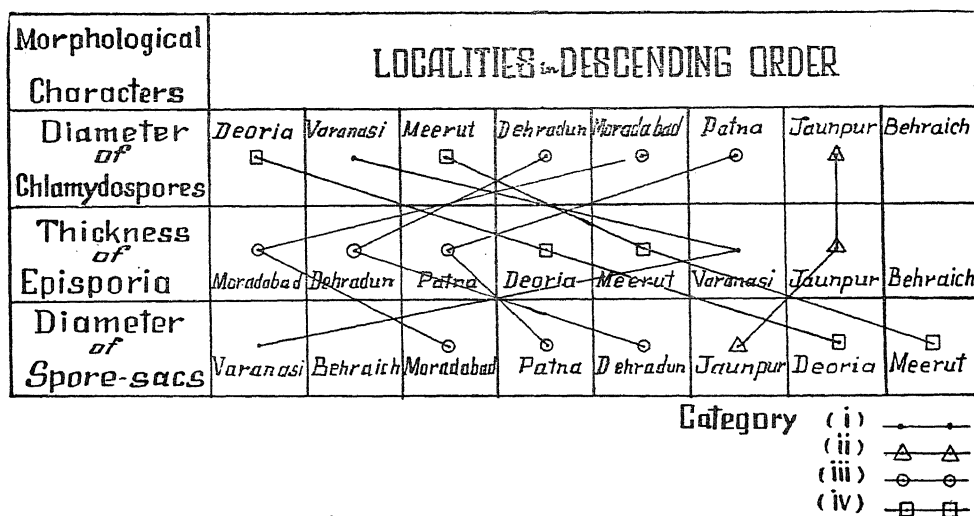
### General consideration :

Earlier workers (Levine, 1928 ; Waterhouse, 1930) have suggested that distinct biologic forms of an organism differ in their quantitative morphological characters as well.

In *Protomyces macrosporus* Ung. collections of chlamydospores from distinctly separated localities indicate differences statistically significant in diameter of their chlamydospores, thickness of episporia and also diameter of germinated spore-sacs.

Before attempting to classify the samples arbitrarily into distinct categories on the basis of any one of the above characters individually, grouping is attempted taking into consideration all the three characters simultaneously. On this basis samples from the localities can be classified into four categories except the samples from Behraich. To note the trends exhibited by each category in relation to all the three morphological characters together a diagrammatic representation has been made in figure 1.

Fig. 1



The following points emerge from fig. 1 :

- (i) Varanasi sample has comparatively longer diameter and thin episporium. On germination these chlamydospores produce larger spore-sacs indicating that the size of the chlamydospores diameter and nature of episporium affect the size of the spore-sac. This trend of bigger chlamydospore with relatively thin episporium and larger spore-sacs is only exhibited by the sample from Varanasi and, therefore, it forms a separate group.
- (ii) Jaunpur collections have smaller chlamydospores with thin episporium and also have smaller spore-sacs. These characters, being quite different from those of the other categories are shown by this sample alone and thus warrant the inclusion of the sample into a second distinct group.
- (iii) Samples from Dehradun, Moradabad and Patna are included in one category. The thickness of the episporium of all the three samples is

greater while the size of chlamydospores and the spore-sacs is intermediate as compared to those in the categories (i) and (ii).

- (iv) The samples from Deoria and Meerut have relatively larger chlamydospore diameters but with small spore-sacs. In this case there seems to be no correlation between the diameter of chlamydospores to that of spore-sacs. The samples showing this trend are, therefore, placed in a separate category.

From the present study, it is evident that two distinct categories exist in relation to variations of the spore characters under observation ; however, a comparison of the three morphologic characters together reveals three or four lines indicating perhaps the possibility of three or four physiologic forms, which have to be confirmed by infection experiments.

### Summary :

The biometric studies on the diameter of chlamydospores, thickness of episporia and diameter of germinated spore-sacs indicate quantitative morphological differences among the samples from different localities. Two distinct categories are noticed on the basis of each morphological character but there is no overlapping of categories. A comparison of the three morphological characters together reveals the possibility of four biological forms existing in the samples of various localities.

### References :

- \*Büren, Gunther Von. Weitere Untersuchungen über die Entwicklungsgeschichte und Biologie der *Protomycetaceae*. *Beilage Kryptogamenflora Schweiz*, 5: 1-94, 1922.
- Dalela, G. G. and Sinha, S. Further studies on biometry of uredospores of *Puccinia penniseti* Zimm. *Proc. Nat. Aca. Sci. India*, 27B: 245-250, 1957.
- Levine, M. N. A statistical study of the comparative morphology of biologic forms of *Puccinia graminis*. *Jour. Agri. Res.*, 24: 539-568, 1923.
- Biometrical studies on the variation of physiologic forms of *Puccinia graminis tritici* and the effect of ecological factors on the susceptibility of wheat varieties. *Phytopath.*, 18(1): 7-123, 1928.
- \*Popta, C. M. L. Beitrag zur Kenntniss der Hemiasci. *Flora*, 86: 1-46, 1899.
- Waterhouse, G. L. Australian rust studies II Biometrical studies on the morphology of spore forms. *Proc. Linn. Soc. N. S. Wales*, 55: 159-178, 1930.

---

\*Originals not seen : Source from Review of Applied Mycology.

# THE GROWTH AND YIELD OF *TRITICUM AESTIVUM* UNDER THE INFLUENCE OF GIBBERELIC ACID

By

AMAR SINGH

Botany Department, Allahabad University

[Received on November 12, 1962]

Application of gibberellins have been known to effect hyper-elongation of plant organs. Beneficial application of gibberellins remains limited and in view of the conflicting scientific evidences further trials are necessary to effectively harness the gibberellins for increased crop production.

*Triticum aestivum*, the growth and yield of which has been investigated and reported herein, has been categorised as unresponsive to gibberellins by Merck and Company workers (1957) and as otherwise by Brian and Grove (1957); I. C. I. workers (1955) and Lona and Bocchi (1956).

Acclimatised seeds of *Triticum aestivum* (C<sub>13</sub>) were sown in a 1 : 1 mixture of Farm-yard manure and soil in earthen pots. Only one plant was allowed to establish and grow in each pot. The plants, when one month old, were treated with gibberellic acid in alcoholic solution, at varying levels of concentration applied as micro drop on the first formed leaf of the main tiller, referred to as treated tiller in the text. The treatments formed application of 0.02 cc of alcoholic solution of gibberellic acid in three doses of 10 (GA-1), 100 (GA-2) and 1,000 ppm (GA-3), besides that of alcohol of similar amount and the control. Alcoholic solution of gibberellic acid was prepared in the dark and stored in coloured bottles as a safeguard against possible chances of deterioration by ultra-violet radiation which has been detected by Yabuta *et al* (1951). The treatment was repeated once, a fortnight after the first application.

Morphogenetic effects, *viz.*, linear growth of treated tiller, number of leaves on the treated tiller, size of leaves, linear growth of first, second, third and fourth internodes of the treated tillers, total number and combined length of all the tillers of plants for any treatment, were made every week and the average considered for evaluating the response. Yield and ear characters were recorded at harvest. The data have been analysed statistically to test the levels of significance of the responses.

## The Findings.

### Linear growth :

*The Treated Tiller* : The treated tiller showed linear growth throughout the period of observation irrespective of the treatments. Of the treatments, maximum increase was observed in the GA-3, followed by GA-2, GA-1, alcohol and control sets of plants at the different stages of observation. On an average, alcohol application did not prove significant at any stage. Maximum increase, at any stage, was observed under the influence of GA-3 treatment over the control. Minimum difference was observed between the control and alcohol treated plants (Table 1).



TABLE 1  
Effect of gibberellic acid on the treated tiller of wheat  
(Average linear growth, cm/plant)

Treatments	Period after first treatment (weeks)			
	1	2	3	4
Control	12.8	23.0	34.6	49.0
Alcohol	14.3	24.6	38.0	48.8
GA-1	21.0	38.3	61.9	65.8
GA-2	25.7	49.6	70.5	74.5
GA-3	24.8	55.8	80.0	81.4
S.E.	1.692	3.102	3.102	3.948
C.D. at 1%	4.674	8.570	8.568	10.908
C.D. at 5%	3.464	6.322	6.350	8.075

*The first Internode* : GA-3 level increased the linear growth of the first internode by 9.9, 11.2, 18.9 and 9.15 cm, over the control in the four-weekly observations. GA-2 increased it to a lesser extent and GA-1 to much less. GA-1 proved insignificant over control at the 2-week stage while the other treatments proved significant (Table 2).

TABLE 2  
Effect of gibberellic acid on first internode of the treated tiller of wheat  
(Average linear growth, cm)

Treatments	Period after first treatment (weeks)			
	1	2	3	4
Control	3.6	5.4	8.3	18.0
Alcohol	4.8	6.0	5.4	18.0
GA-1	5.6	12.2	17.8	23.1
GA-2	10.4	13.4	23.6	25.1
GA-3	10.0	13.8	25.7	27.5
S.E.	3.243	1.833	1.551	1.833
C.D. at 1%	8.950	5.064	4.285	4.064
C.D. at 5%	6.758	3.753	3.176	3.753

At the 2-week stage the effect of increasing levels of GA application was not as marked as at other stages. The first slab of 10 ppm GA indicated greatest response in linear growth over the control than subsequent raising of level of GA over the preceeding doses. At subsequent stages also the effect was manifested.

*The Second Internode* : The effect of gibberellic acid on the linear growth of the second internode of the treated stem was significant with certain exceptions.

GA-1 level failed to affect a significant increase for the first three weeks though at the 4th week stage it proved significant over the control (Table 3).

TABLE 3  
Effect of gibberellic acid on the second internode of the treated tiller of wheat  
(Average linear growth, cm)

Treatments	Period after first treatment (weeks)			
	1	2	3	4
Control	3.0	8.1	9.3	10.7
Alcohol	3.1	7.2	10.0	10.4
GA-1	4.5	10.8	13.4	14.0
GA-2	7.5	18.2	13.6	13.9
GA-3	6.6	19.5	19.0	17.9
S.E.	1.099	3.102	0.338	1.099
C.D. at 1%	2.936	8.570	0.933	3.034
C.D. at 5%	2.250	6.352	0.632	2.250

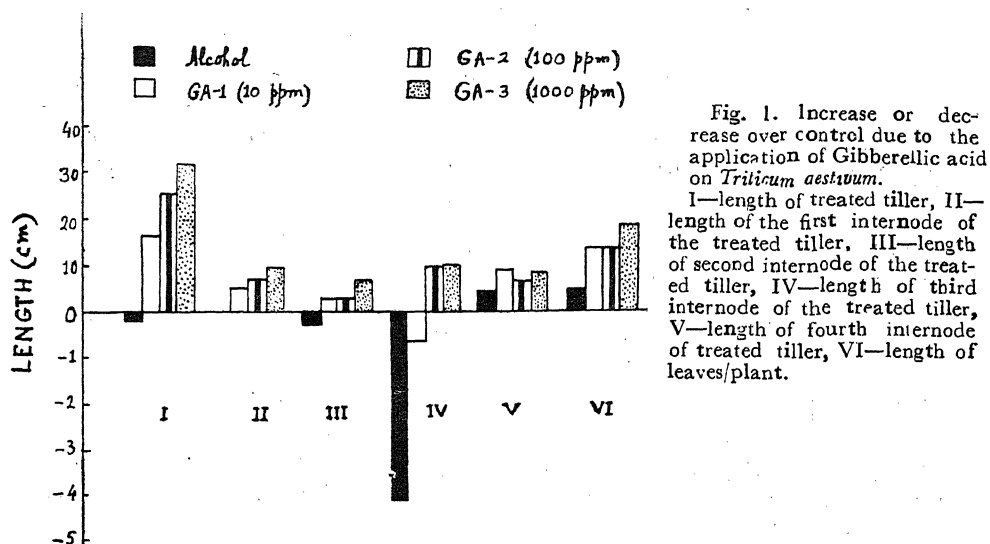
GA-2 and GA-3 treatments were significant at all the stages in affecting the linear growth of the second internode (Fig. 1). The application of alcohol did not produce beneficial results.

*The Third Internode* : Gibberellic acid, in all the concentrations, proved insignificant in the first week towards linear growth of third internode of the treated tiller (Table 4).

TABLE 4  
Effect of gibberellic acid on third internode of the treated tiller of wheat  
(Average linear growth, cm)

Treatments	Period after first treatment (weeks)			
	1	2	3	4
Control	5.6	7.5	8.3	12.0
Alcohol	5.6	7.3	7.0	7.9
GA-1	5.0	13.6	11.2	11.4
GA-2	5.7	12.7	18.5	21.4
GA-3	6.6	17.4	19.7	21.5
S.E.	Insignificant	2.256	1.128	2.397
C.D. at 1%		6.233	2.116	6.622
C.D. at 5%		5.620	3.309	5.728

GA-1 and GA-2 proved deleterious and GA-3 only slightly superior to control at this stage. GA-1 proved better in the second week and thereafter insignificantly so. Of the GA concentrations GA-3 at the 2-week stage and GA-2 and also GA-3 at the 3-week as well as 4-week stage proved significantly superior over its preceeding dose. 4 weeks after the start of the experiment alcohol proved highly deleterious (Fig. 1).



*The Fourth Internode*: The fourth internode of the treated stem increased in length with age as well as the application of gibberellic acid. Increase in dosage of GA worked significantly towards linear growth of the internode (Table 5).

TABLE 5  
Effect of gibberellic acid on fourth internode of treated tiller of wheat  
(Average linear growth, cm)

Treatments	Period after first treatment (weeks)		
	1	2	3
Control	2.8	8.7	8.3
Alcohol	5.4	10.4	12.5
GA-1	9.7	13.0	17.3
GA-2	8.2	13.6	14.1
GA-3	10.4	13.0	14.5
S.E.	1.692	1.692	2.679
C.D. at 1%	4.653	4.582	7.402
C.D. at 5%	3.465	3.464	5.487

Alcohol application did not prove of any significance on this score at any stage. At the last observation GA-1 was most effective followed by GA-3, GA-2 and alcohol in succession (Fig. 1).

*Total Tiller Length*: Length of all the tillers, for any treatment, when taken together, was affected significantly by gibberellic acid at all the stages. The effect of GA-1 over control was more marked than that of GA-2 over GA-1 or GA-3 over GA-2 in the first and second weeks though not in the third week. The changes in the GA level from GA-1 to GA-2 did not prove significantly effective in increasing the total length of the tillers (Table 6).

TABLE 6  
Effect of gibberellic acid on wheat tillers  
(Average total length, cm/plant)

Treatments	Period after first treatment (weeks)		
	1	2	3
Control	54.4	82.6	149.0
Alcohol	53.6	102.3	165.0
GA-1	74.3	150.0	219.7
GA-2	80.0	163.6	244.3
GA-3	98.0	220.6	333.7
S.E.	5.399	14.664	21.996
C.D. at 1%	14.917	40.516	50.774
C.D. at 5%	11.056	30.031	45.047

Alcohol application was never significantly effective, though in the second and third weeks it did bring about slight increase in length. Four weeks after the first application of gibberellic acid the alcohol treatment showed minimum effectiveness while maximum was shown by GA-3 treatment (Fig. 1).

*Tillering Capacity*: Tillering capacity of the plant did not increase significantly under the influence of any of the treatments (Table 7).

TABLE 7  
Effect of gibberellic acid on tillering in wheat  
(Total no./plant)

Treatments	Period after first treatment (weeks)			
	1	2	3	4
Control	10.0	12.8	13.2	13.4
Alcohol	9.4	14.6	16.7	16.8
GA-1	10.7	12.3	13.6	13.7
GA-2	10.8	12.0	12.5	12.9
GA-3	10.9	13.3	13.4	13.5

Responses insignificant.

The effect of alcohol applied alone, proved deleterious though insignificantly in the first week, later it increased tillering slightly over other treatments.

*Leaf Formation* : The trend of the effect of gibberellic acid on the number of green leaves present on the treated tiller is depicted in Table 8. The treatment effects are insignificant and irregular throughout the period of observation.

TABLE 8  
Effect of gibberellic acid on green leaves in the treated tiller of wheat  
(Average no./tiller)

Treatments	Period after first treatment (weeks)			
	1	2	3	4
Control	4.0	3.0	4.3	3.9
Alcohol	3.6	3.9	4.7	4.0
GA-1	3.7	4.6	4.8	4.7
GA-2	3.7	3.6	4.3	4.8
GA-3	3.6	4.0	4.0	4.4

Responses insignificant

*Leaf Expansion in Length* : The length of the leaves increased with age and also under the influence of some of the treatments. The effect of the treatments as well as of age was significant one week after the treatment (Table 9).

TABLE 9  
Effect of gibberellic acid on expansion of leaves of the treated  
tiller of wheat  
(Average linear growth, cm/tiller)

Treatments	Period after first treatment (weeks)			
	1	2	3	4
Control	76.0	75.0	94.7	85.0
Alcohol	72.6	77.3	107.0	89.8
GA-1	80.8	98.4	111.9	90.1
GA-2	80.4	97.9	104.0	98.9
GA-3	97.6	104.8	111.7	103.6
S.E.	Insignificant	9.306	5.217	5.399
C.D. at 1%	Response	25.712	14.414	14.917
C.D. at 5%		19.058	10.684	11.056

GA-1 treatment proved more effective than the GA-2 which seemed not to be effective in increasing leaf length. The GA-3 treatment brought forth maximum increase at the 3 week stage after which it showed a decline,

*Leaf Expansion in Breadth*: In the matter of leaf breadth the application of GA at 1,000 ppm proved markedly increasingly effective over GA-1 (100 ppm) at the two weeks stage. At this age the GA-2 treatment also proved efficient in increasing leaf breadth over the control (Table 10).

TABLE 10  
Effect of gibberellic acid on the treated tiller of wheat  
(Average breadth of leaves, cm/tiller)

Treatments	Period after first treatment (weeks)			
	1	2	3	4
Control	4.0	4.9	6.7	5.5
Alcohol	3.6	4.6	6.6	5.3
GA-1	3.9	5.6	6.8	5.6
GA-2	3.6	5.9	6.3	5.4
GA-3	3.5	5.8	5.7	4.8
S.E.	Insignificant	0.310	0.296	Insignificant
C.D. at 1%		0.857	0.794	
C.D. at 5%		0.635	0.606	

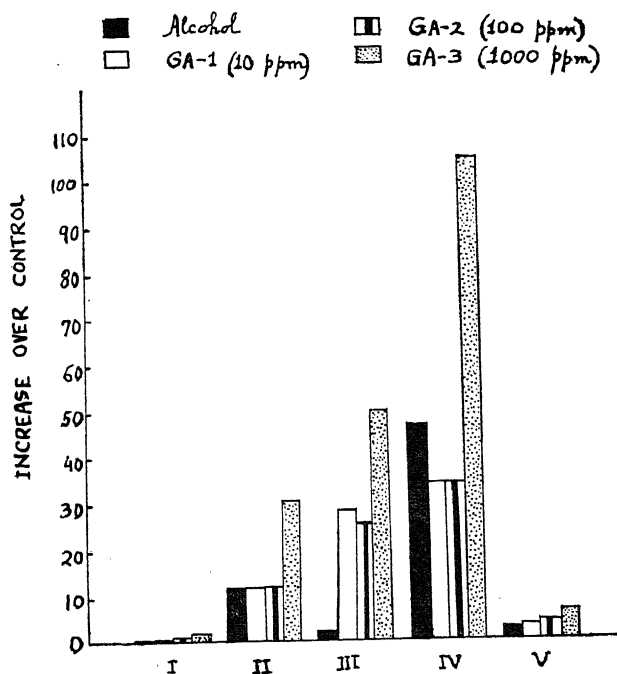


Fig. 2. Increase or decrease over control due to the application of gibberellic acid on *Triticum aestivum* plant as observed at harvest. I—length of ear on treated tiller, II—number of spikelets on treated tiller, III—total number of spikelets on all the ears/plant, IV—number of grains/plant, V—dry weight of stem/plant

A week later GA-1 proved significant in this respect but none else. In the first and fourth weeks all the treatments remained insignificant without exception.

*Response at Harvest :* Of the observations made at harvest, length of the ear of the treated tiller, number of spikelets per plant, combined length of all the ears, weight of stem and number of grains per plants, were affected significantly by GA (Table 11).

TABLE 11  
Effect of gibberellic acid on wheat at harvest  
(Per plant basis unless specified)

Treatments	Ear length/ treated tiller (cm)	Spikelets no./treated tiller	Spikelets (total no.)	Grain no./ treated tiller	Ear no.	Total ear length (cm)	Stem weight (cm)	Grain (no.)	Grain weight (gm)
Control	8.5	21.6	136.3	46.0	7.2	50.0	7.6	243.3	8.1
Alcohol	8.7	19.5	138.0	43.6	8.9	61.3	9.9	290.7	10.7
GA-1	9.1	22.3	164.6	45.6	8.6	61.6	10.0	276.6	10.9
GA-2	9.7	22.8	161.7	46.6	8.6	60.9	11.3	277.6	10.9
GA-3	10.2	21.5	186.0	47.5	10.5	79.0	14.5	247.8	14.4
S.E.	0.045	N.S.	13.818	N.S.	N.S.	4.653	1.663	27.213	N.S.
C.D. at 1%	1.146		38.149			11.856	4.574	75.189	
C.D. at 5%	0.923		28.289			9.529	3.404	55.732	

The level of effectiveness increased with increase in GA level for these growth attributes. The growth attributes which could not be significantly affected were number of spikelets, number of grains per treated tiller, number of ears and also weight of grains. To sum up the ultimate significant responses of the two applications of gibberellic acid as existing a fortnight after the second application reference may be made to Fig. 2 depicting the increase or decrease over control.

#### Discussion :

The application of gibberellic acid at 10, 100 and 1,000 ppm caused a significant increase in length of several parts of wheat plant (Fig. 1). The enhancement in linear growth of the tiller was owing to the increase in length of the internodes. Generally, with some exceptions, the response increased with the concentrations applied. Individual internodes contributed to the extension of growth of the plants. It was noticed that all the internodes of the treated tiller were affected significantly thereby suggesting that GA did not show polar movement. There was response from the plant below the site of application as also noted by Yabuta and Hayashi (1939), though not by Yabuta *et al* (1941). The higher concentrations of gibberellic acid had a more pronounced effect on the first internode than on the fourth internode in conformity with the observations of Hillman and Purves (1961) who observed increased elongation of the portion of the stem nearer the tip, while those away from the apex exhibited lesser growth consequent to the application of growth substance. The findings seemed to suggest that more apical the internode, the greater was the response to gibberellic acid possibly on account of their higher endogenous auxin content. It supported the contention that gibberellic acid caused sparing of auxin and that its action was auxin-mediated.

Examining the response of gibberellic acid to the number and length of tillers it was observed that while there was no effect on the tillering capacity, tiller length did increase (*cf.* Tables, 6, 7). Such a selective action could be explained by the findings of Rao *et al* (1960) with sugarcane, to the effect that the metabolites instead of being utilised for tillering were used up in shoot elongation under the action of gibberellic acid. Increased dry matter production of the elongated tiller should have been the outcome but such an effect was not observed by Morgan and Mees (1958). They found that grain and straw yield of wheat were not increased in spite of enhanced vegetative growth of the plants. In the present findings grain yield did not increase significantly though stem weight increased significantly by GA application. The assimilatory leaf surface area increased by GA application definitely at the 3-week stage.

Leaf length increased significantly with 1,000 ppm dose of GA specially at the two later stages of observations (Table 9). These observations are in conformity with those reported by Brian (1958) who obtained leaves twice their normal length as a result of application of GA. Leaf expansion was not increased under all the gibberellin concentrations as also reported by Yabuta and Hayashi (1939) who observed that in tomato, morning glory and three cucurbits leaf expansion was inhibited, but at one gibberellin concentration cucumber leaf expansion was promoted.

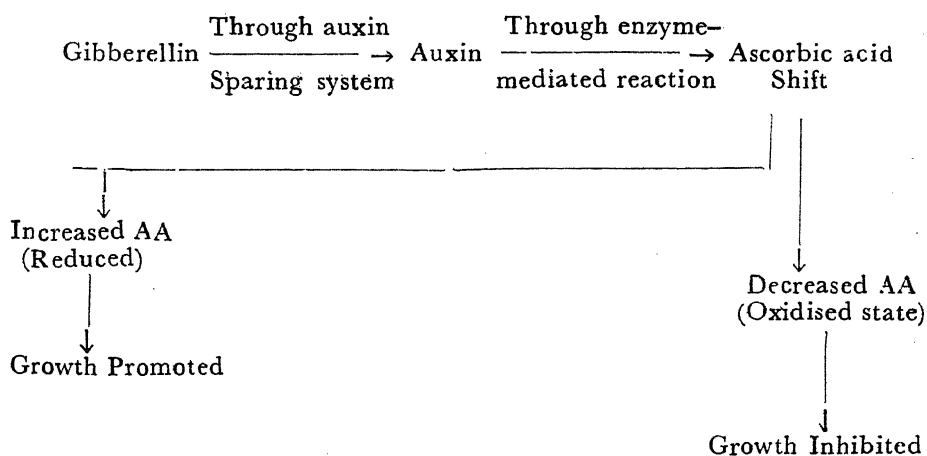
The action of gibberellic acid on the morphological expression of wheat plant during its life cycle has been quite spectacular in linear growth (Fig. 1). The number of grains in the plant increased significantly though not its weight (Table 11). Enhancement of vegetative growth accompanied by increase in yield has not been recorded by Morgan and Mees (1958) and Wittwer and Bukovac (1958). In so far as the increase of the total dry matter production was concerned the findings are in line with those of Scurfield and Biddiscombe (1958).

Gibberellic acid increased growth of internodes, possibly, through its action on the metabolism especially of the meristematic cells *via* RNA and DNA, that controlled protein synthesis, played a prominent role (Sironval, 1961). The application of auxin to tobacco pith callus culture induced an increase in the DNA and RNA content of the cells. The observations of Sachs (1959) indicated that gibberellic acid could act as a potent factor in cell division leading to an increase in nucleic acid as the outcome of the gibberellic acid action. Further, the increase in the level of ascorbic acid following application of gibberellic acid (Mosolov and Mosolova, 1959) might be a cause of increased growth since stimulation of respiration (Bourdeau, 1958) accompanied by release of energy and ATP occurred.

The relationship of ascorbic acid content of the plant to its growth was further confirmed by Key (1962), who held that the net effect of auxin application was the accumulation of dehydroascorbic acid in the stem leading to increased growth. Humphreys *et al* (1957*a*, 1957*b*, 1959) and also Black and Humphries (1960) have recorded the stimulation of pentose phosphate pathway activity by auxin application. Such an activation was known to produce TPNH which was utilized to reduce oxidized glutathione and thus dehydroascorbic acid (Veenesland and Conn, 1954). Greater activation of pentose phosphate pathway led to an enhanced production of TPNH in the stem bringing about a more reduced state of glutathione and ascorbic acid that caused an increase in linear growth of the stem.



A tentative scheme of the nature of the action of gibberellic acid on the growth of plants may thus be postulated as under :



#### Summary :

The effect of gibberellic acid applied at 10, 100 and 1,000 ppm concentrations in alcohol solution to the first leaf of the main tiller, at the age of one month and again a fortnight after, of *Triticum aestivum* (C<sub>13</sub>) plants grown in parts with 1 : 1 mixture of soil : farmyard manure was evaluated statistically. GA treatment affected elongation of treated tiller as well as its internodes, total length of all the tillers, number of leaves on the treated tiller (after the second application), leaf length and leaf breadth (at certain stages), ear length of the treated tiller, total number of spikelets, total ear length, stem weight and grain number significantly while number of tiller, number of leaves, grains/tiller, and spikelets of treated tiller remained unaffected. On the basis of the morphogenetic developments of the plant a tentative scheme of gibberellic acid action has been postulated.

#### Literature Cited :

- Black, C. C. and Humphries, T. F. *Plant Physiol. Suppl.*, **35** : 27, 1960.  
 Bourdeau, P. F. *Nature*, **182** : 118, 1958.  
 Brian, P. W., Eton, G. W., Hemming, H. G. and Radley, M. *Jour. Sci. Food and Agr.*, **5** : 602-612, 1954.  
 Brian, P. W. and Grove, J. F. *Endeavour*, **XV**<sub>1</sub> (63) : 161-171, 1957.  
 ——— The Fernhurst Lecture. *The Royal Soc. Arts*, London, 1958.  
 Hillman, W. S. and Purves, W. K. *Fourth Int. Cont. Plant Growth Regulation*, Iowa State Publication, 589-600, 1961.  
 Humphreys, T. E. and Duggar, W. M. *Plant Physiol*, **32** : 136-140, 1957a.  
 ——— *Ibid*, **32** : 530-536, 1957b.  
 ——— *Ibid*, **34** : 580-582, 1959.  
 Imperial Chemical Industries Ltd. *New Organic Compounds Patent Appln.* Care 11504/11656A (Commonwealth) Australia, **28** : 6.55, 10190.  
 Kato, J. *Science*, **123** : 1132, 1956.  
 Key, J. L. *Plant Physiol*, **37** : 349-356, 1962.

- Lona, F. and Bochhi, A. Source Book on Gibberellin, *Abs.* 261, 1956.
- Merck & Co. Inc. Gibrel.—A Progress Report, 1957.
- Morgan, D. G. and Mees, G. C. *J. Agri. Sci.*, **50** : 49-59, 1958.
- Mosolov, I. U. and Mosolova, L. V. *Izv. Acad. Nauk SSSR*, **4** : 579-589, 1959.
- Rao, G. N., Rao, N. V. M. and Rao, P. H. *Ind. Jour. Sug. Res. and Dev.*, **4** (3) : 144-148, 1960.
- Sachs, R. M., Bertz, C. and Lang, A. *Amer. Jour. Bot.*, **46** : 376-384, 1959.
- *Exper. Cell. Res.*, **18** : 230-244, 1959.
- Scurfield, G. and Biddiscombe, E. F. *Nature*, **183** : 1196-97, 1959.
- Sironval, C. Fourth Int. Symp. Plant Growth Regulation. Iowa State University, 521-530, 1961.
- Veensland, B. and Conn, E. E. S. Colowick et al eds. *Acad. Press, Inc. New York* 105-127, 1954.
- Wittwer, S. H. and Bukovac, M. J. *Econ. Botany*, **12** : 213-55, 1958.
- Yabuta, T. and Hayashi, T. *J. Agr. Chem. Soc. Japan*, **15** (175) : 403-13, 1939.
- Yabuta, T., Sumiki, Y., Tamura, T. and Murayana, N. *Ibid.*, **17** (12) : 1001-1004, 1941.
- Yabuta, T., Sumiki, Y., Fukamufa, K. and Horiuchi, M. *Ibid.*, **24** (8) : 395-396, 1951.

# CONTROL OF LOOSE SMUT OF BARLEY—BY SEED TREATMENT

By

R. S. MATHUR

*Plant Pathologist to Government, Uttar Pradesh*

and

J. SWARUP

*Bacteriologist, Section of the Plant Pathologist to Government, Uttar Pradesh, Kanpur*

[Received on July 31, 1963]

Loose smut of barley [*Ustilago nuda* (Jens.) Rostr.] being an internally seed-borne disease is not ordinarily controlled by fungicidal seed treatments. However, appreciable control of the disease was reported by presoaking the seed in water for 6 hours and subsequently dipping it in a suspension of Spergon SL for different periods of time (7, 8, 9, 10). Water soak treatments (1, 7, 10), anaerobic treatments of seed in air-tight containers (3, 4, 5, 8, 12) and solar heat treatments (2, 6, 11) also proved promising. Further studies on anaerobic treatments and presoaking of barley seed for the control of loose smut are reported in this paper.

Seeds of barley varieties K.12 and K.19 were first inoculated artificially with freshly collected spores of *Ustilago nuda* by the Moore's partial vacuum method; then they were given the following treatments at room temperature (average 19°C): (1) seeds soaked in water for 6 hours, then dipped in 0.2% suspension of Spergon SL (tetrachloro-parabenzo-quinone) for 24 and 48 hours; (2) seeds were soaked in sufficient quantity of tap water for 48, 60 and 72 hours; (3) seeds presoaked in water for 2 hours were held in air-tight bottles, occupying half of its volume approximately, for 48, 60 and 72 hours; (4) seeds presoaked in water for 6 hours at room temperature were kept first in water maintained at 50°C for 5 minutes and then in water maintained at 54°C for 10 minutes. One hundred treated and untreated seeds were planted 4" apart in two rows of 16 ft. each. Each treatment was randomized and replicated three times in a factorial design.

Germination of seed after sowing and infection of ears in adult plants were recorded, and the data were transformed into angles ( $\text{angle} = \sin^{-1} \sqrt{\text{Percentage}}$ ) and analysed statistically. There was no significant variation in germination of the seed due to treatments, varieties and their interaction. The variation in the percentage smut infection due to treatments was highly significant whereas the variation due to varieties, and interaction (treatments  $\times$  varieties) were not significant. (Table 1).

From the summary of results given in table 1, it is evident that all treatments are significantly superior to control. Hot water treatment is significantly superior to other treatments except that it is not different from presoaking of seed in water at room temperature for 72 hours. Although the treatments of water soaking for 72 hours, spergon for 48 hours, water soaking for 60 hours and anaerobic treatment for 72 hours are not significantly different among themselves yet they are significantly superior to treatment of seed by spergon suspension for 24 hours.

TABLE 1  
Effect of presoak and anaerobic treatments on the control of  
*Ustilago nuda* causing the loose smut of barley

Treatmenets	Average Angle $\text{Sin}^{-1} \sqrt{\text{Percentage}}$	Average percentage Infection (Transformed back)
Hot water	0.00	0.00*
Water soaking 72 hours	4.46	1.09
Spergon 48 hours	10.23	3.92
Water soaking 60 hours	10.39	3.72
Anaerobic 72 hours	10.45	3.76
Anaerobic 48 hours	12.03	4.80
Anaerobic 60 hours	15.63	7.69
Water soaking 48 hours	16.58	8.62
Spergon 24 hours	18.67	10.64
Control	29.18	24.16
General Mean	12.75	5.32
C.D. at 1% level	10.04	
C.D. at 5% level	7.28	

\*Bias correction not added.

#### Acknowledgement :

Thanks are due to Mr. G. K. Bajpai for statistical analysis of data and to Dr. S. C. Mathur for critically reading the manuscript.

#### References :

- Arny, D. C. and Leben, C. Control of several small grain diseases by the water-soak method. (Abs.). *Phytopathology*, **44** : 481, 1954.
- Bedi, K. S. Control of loose Smut of barley with Solar-heat in the Punjab plains. *Indian Phytopath.* **10** (2) : 138-141, 1957.
- Hebert, T. T. A new method of controlling loose smut of barley. *Plant. Dis. Repr.*, **39** : 20-22, 1955.
- Mode of action of the wet anaerobic storage treatment for the control of loose smut of barley. (Abs.). *Phytopathology*, **46** : 14, 1956.
- Minz, G. and Gavrielith-Galmond, H. An anaerobic method for the control of loose smut of barley. *Hassadeh*, **39** (6) : 622, 1959 [Abs. *Rev. appl. Mycol.* **40** : 42, 1961].

- Patel, M. K., Dhande, G. W. and Kulkarni, Y. S. A modified treatment against loose smut of wheat. *Curr. Sci.*, **19** (10) : 324-325, 1950.
- Russel, R. C. and Tyner, L. E. The influence of temperature on the time required to control loose smut of barley by means of the Spergon or water-soak treatments. *Can. J. agric. Sci.*, **34** : 533-538, 1954.
- Tandon, I. N. and Hansing, E. D. Control of loose smut of barley by water-soak and anaerobic treatments. *Plant Dis. Repr.*, **41** (3) : 202-204, 1957.
- Tyner, L. E. Control of loose smut of barley by chemical and physical treatments. *Sci. Agr.*, **31** : 187-192, 1951.
- The control of loose smut of barley and wheat by Spergon and soaking in water at room temperature *Phytopathology*, **43** : 313-316, 1953.
- Vasudeva, R. S. and Iynengar, M. R. S. Control of loose smut of barley. *Curr. Sci*, **19** (7) : 218-219, 1950.
- Zemanek, I. and Bartosh, P. A new method for treating barley seed against loose smut. *Mezh. sel.-Khoz. Zh.*, **4** (3) : 81-89, 2 fig., 1960. [Abs. *Rev. appl. Mycol.*, **40** : 100, 1961].

# THE ORIGIN AND UTILITY OF SOME VERNACULAR PLANT NAMES\*

By

S. K. JAIN

*Botanical Survey of India, Calcutta*

[Received on September 7, 1962]

## Introduction :

Plants are generally known by their local names. Binomial Latin names for plants are used in scientific literature. Most of the knowledge about plants has passed from one generation to another chiefly through local plant names. People know indigenous plants of their region for one or more of the following reasons :

- (a) for their use as source of food material ;
- (b) for their use as timber ;
- (c) for their use in medicine ;
- (d) for use as fuel ;
- (e) for use in beverages and narcotics ;
- (f) for several other uses such as fodder, fibre, dye, tan, oil, gum, perfume, etc. ;
- (g) for use in witch-craft ;
- (h) for worship and association with mythology ;
- (i) for poisonous effects on human beings and cattle ;
- (j) being nuisance as weeds ;
- (k) and for several inconveniences caused by them due to their thorns, spines, acrid juices, obnoxious odour or even frightful appearance.

Plants having some relationship with everyday life of people are, thus, constantly required to be referred and are assigned vernacular names. Only some less important or rare plants are not given any local names.

The main reason for too many local names for plants in our country is our agricultural economy. Over eighty percent of Indian population resides in villages and about seventy percent people depend on agriculture. They live in close vicinity of plants. In olden days, when large-scale industry was less or even none, people wholly depended for their livelihood on the product of their vicinity. All demands of life were met compulsorily from the natural products of the region, chiefly the plant wealth. Large number of plants were, thus, used for meeting one requirement or the other, and people developed an intimate relationship with plants. All plants known for one reason or another were given local names.

A local name often describes some characteristic feature of the plant or the plant part in which the community is interested. The descriptive word to denote

---

\*This paper is from the series 'Studies in Ethnobotany of India'.

that character is different in different languages or dialects and, hence, different names are assigned to the same plant in different languages and regions.

Further, the relation of a plant with people in one community may be different from the other. Same plant may be considered useful by some people but injurious by others. In such cases, the names assigned to it by two people even of same language or region may vary.

These factors result in too many local names for one plant. Kirtikar and Basu (1935) have listed about one hundred local and common names for *Melia azedarach*. Several hundred plants have over twenty local names.

#### Service rendered by local names :

- (i) The local names have rendered some very useful service. They were the earliest means of referring to plants and, it is through their agency that most of the knowledge about plants has come to us from our ancestors.
- (ii) The form of local names conforms to the local language or dialect and hence their pronunciation is simple as well as easy for people of the region to remember.
- (iii) Very often the local names refer to some salient feature in shape, size or utility of the plant and such qualifying names can be a source of useful information about these plants. Some interesting examples of these qualifying names were noted while working among the aboriginal tribes of Madhya Pradesh, particularly in Bastar district.

##### (a) names referring to shape :

*Martinia annua* is called *Baghnakha* (*Bagh* : tiger ; *nakha* : nails) due to the shape of the fruit resembling the nails of a tiger (fig. A). The plant is considered medicinal. *Bryonopsis laciniosa* is called *Shivlingi*. The name refers to the shape of seeds resembling the idol of 'Shivalinga' worshipped in many parts of India (fig. B). The plant is medicinal. *Leonotis nepetaefolia* is called *Barchhi-but* (*Barchhi* : spear, lance ; *but* : plant). The name refers to spear-like appearance and prickly nature of the inflorescence (fig. C). Seeds are given in disorders of urination, and to cattle in dropsy. *Helicteres isora* is named *Marorphali* of *Ainthe-gainthi*. (*Maror* and *Ainthe* mean twist ; *phali* means fruit). The name refers to the twisted fruit (fig. D). The fruit is useful in griping pain of bowels. The griping pain is locally called *Marora* or *Ainthan*. The name may as well be referring to its property of curing the *Marora* or *Ainthan* disease. *Nelsonia campestris* var. *vestita* is called *Punjki*. (*Punj* : cluster), because the plant has clustered roots (fig. E). Pills made of pasted roots are given to children, suffering from fever, in their mother's milk.

##### (b) names referring to colour :

*Casearia graveolens* is called *Swarna-mula*. (*Swarn* : golden ; *mul* : root). The name refers to golden colour of roots. Bark of root is used as tonic in anaemic conditions.

##### (c) referring to taste :

*Scoparia dulcis* is called *Mithi-patti* (*Mithi* : sweet ; *patti* : leaves) because the leaves, taste sweetish. Leaves, pounded and made into pills, are given in sexual weakness.

(d) referring to medicinal properties :

*Equisetum debile* is called *Had-juri* (*Had* : bone; *juri* : join). There is general belief among these tribals that the broken bones, if tied round by the stems of this plant, get joined up. *Ichnocarpus frutescens* is named *Dudhiakand* (*Dudhia* : milky; *kand* : root). The name refers to the supposed property of the roots of promoting flow of milk in women. *Smilax prolifera* is named *Mutri-laha* (*Mutra* : urine). The aborigines believe that children who suffer from the habit of wetting at night, if given food on the leaves of this plant, are cured.

(e) referring to other uses :

*Mallotus philippensis* is called *Sendur-rukh* (*Sendur* : vermilion ; *rukh* : tree). The red powder obtained from the red glandular covering on the fruits is used by women for applying on their foreheads as vermilion.

*Abutilon indicum* is called *Jhapi-pak* (*Jhapi* : ear-ring). The fruits of the plant are worn by aboriginal women as pendant ear-rings, (fig. F).

*Phoenix acaulis* is named *Chhindi-kanda* (*Chhindi* : name of an insect ; *kanda* : root). An edible, much-sought-for insect, called *Chhindi* lives in the roots of this small palm.

*Thysanolaena maxima* is called *Phulbuhari* (*Phul* : flower ; *buhari* : broom-stick). Broomsticks are prepared from the large inflorescence of this tall grass. It is a cottage industry in several tribal villages.

These few examples will show how precise information the local names sometimes give regarding structure or properties of plants. Numerous such examples are available.

(iv) The aboriginal tribes living in forest areas even today depend for their demands of life on plant products of their vicinity and assign names to such plants as are useful to them. Much useful anthropological information on composition and migration of tribes can be had from an analysis of the plant names used by people in a tribal community today.

(v) The root-word of a plant name and its derivative forms can throw light on development of languages and dialects of the region. In some cases, the several names of a plant are derivatives or slight modifications of one root-word. Such examples are the names of *Azadirachta indica* (*Nim*, *Nimba*, *Limbra*, *Limba*, *Kadulimb*, *Balantnimb*); *Mimusops elengi* (*Bakul*, *Bakulam*, *Bakula*, *Vakulam*); and *Santalum album* (*Chandan*; *Chandana*, *Chandanam*, *Chandanamu*, *Safed Chandan*).

How a plant could be called by the same name or very closely resembling names in far off places? The answer will require a probe into very old literature and early history of people. If the earliest name ever given to a plant could be discovered, a study of later names may indicate the routes followed by that plant or by its commercial products.

(vi) In forest utilisation, an adequate knowledge of local plant names is helpful, rather necessary, for communication of instructions to local labour engaged on extraction of timber and minor forest produce. The same applies to eradication of weeds in agricultural lands.



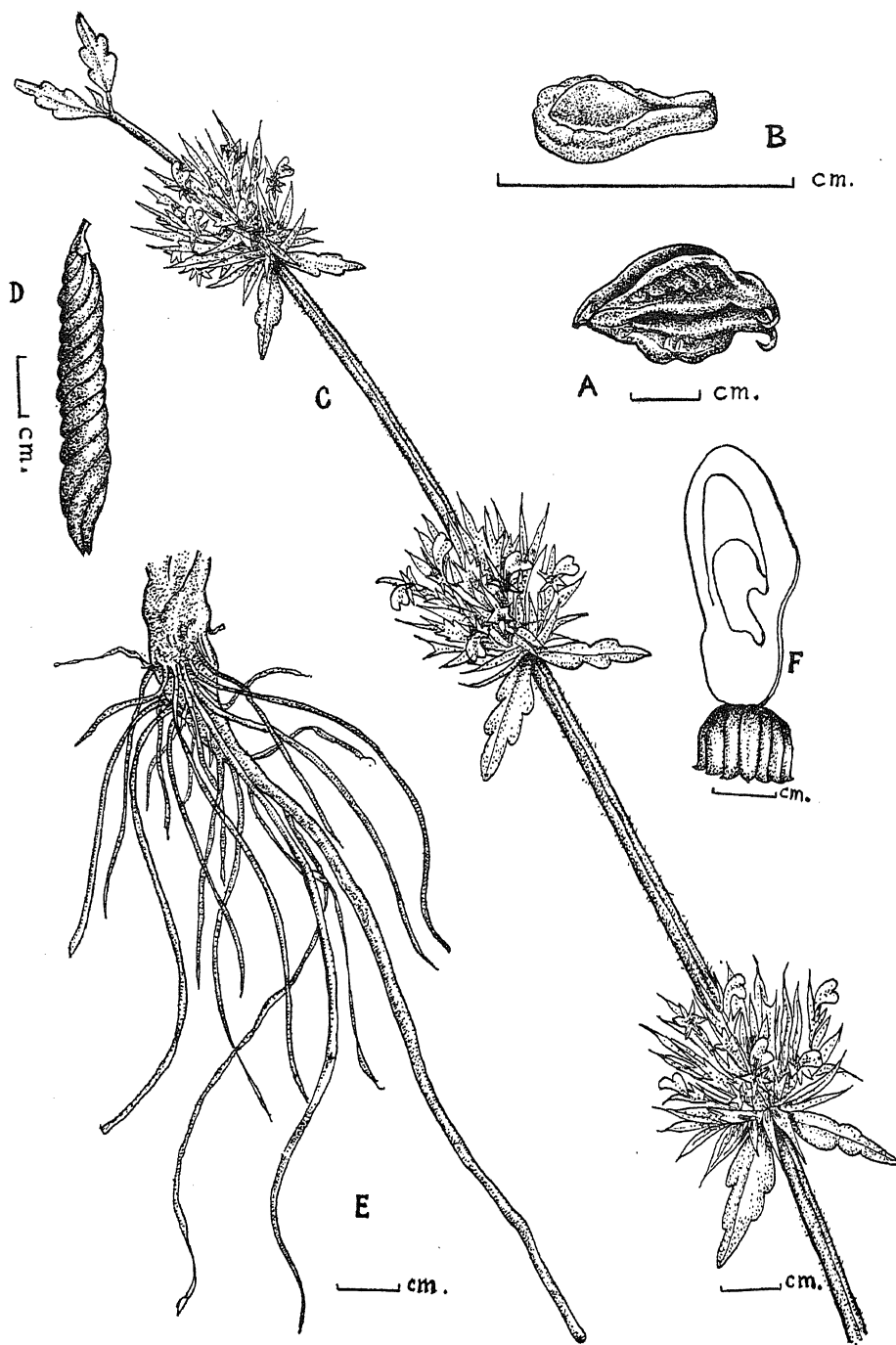


Fig. A. A fruit of *Martinia annua*.  
 Fig. B. A seed of *Bryonopsis laciniosa*.  
 Fig. C. Upper part of inflorescence of *Leonotis nepetaefolia*.  
 Fig. D. A fruit of *Helicteres isora*.  
 Fig. E. Roots of *Nelsonia campestris* var. *vestita*.  
 Fig. F. Fruit of *Abutilon indicum*, used as pendant earring.

## Demerits of local names :

A knowledge of some significant disadvantages of local names makes a field worker careful and cautious.

- (i) Sometimes several different plants are given one and the same local name, e.g., name *Brahmi* refers to *Centella asiatica* and *Bacopa monnieri*; name *Rudanti* refers to *Capparis mooni* as well as *Cressa cretica*. Such examples can be multiplied.
- (ii) One plant is given too many names and there are instances of even a hundred names for one plant. This causes confusion in referring to the plant.
- (iii) Some local names are very misleading. For example: *Harra* is *Terminalia chebula* (Combretaceae). Another tree, whose fruits resemble in shape with *Harra* but the tree is larger, is called *Gaj-harra* (*Gaj* : elephant). This is *Melia composita* (Meliaceae), even a different plant family *Azadirachta indica* (Meliaceae) is commonly known as *Nim*; *Mahanim* is the name for *Ailanthus excelsa* (Simarubaceae), *Akasnim* for *Millingtonia hortensis* (Bignoniaceae), and *Jalnim* for *Bacopa monnieri* (Scrophulariaceae) as well as for *Lycopus europeus* (Labiatae). Thus, derivatives from one root-name *Nim* have been assigned to five plants belonging even to different families. The reason seems to be the common character of bitterness of the leaves in all these species.
- (iv) Sometimes different stages of growth of a plant are given different names. *Eclipta prostrata*, when in flower is called *Shwet-bhangra* (*Shwet* : white); and when in fruit, it is called *Kala-bhangra* (*Kala* : black). The reason is that (in flower) the ray florets have white corolla, and (in fruit) the colour of achenes is black. Some species of *Heliotropium* are also called *Safed-bhangra*.
- (v) Some local names are mono-syllabic, some di-syllabic and others tri- or poly-syllabic.

The demerits of local names as given under (i) and (ii) above are their main defects. Too many local names for one plant or, one and same name for several different plants, both anomalies eliminate their usage in scientific literature.

Other kinds of anomalies (iii to iv) sometimes occur in Latin names too, due to error of someone at some stage. Two common Indian pluses *Mung* and *Urid* belong to the genus *Phaseolus*. The botanical name for *Urid* is *Phaseolus mungo* and for *Mung* it is *Phaseolus aureus*.

An interesting instance of one and same plant being assigned, at its different stages of growth, two different Latin names was discovered by Santapau (1945) in the genus *Curcuma*.

Botanical names, which are now uniformly binomial, used to be trinomial or polynomial only about two hundred years ago.

Thus, whereas, for purposes of universal usage or reference, the local name is not a competitor to the scientific name, and the Latin binomial names are essential for uniformity and consistency, the local names too have much to be said in their support for purposes of restricted reference work.

A proper record and appreciation of vernacular plant names can be useful for many branches of knowledge, particularly to the sciences of botany, medicine, cultural anthropology, comparative philology, forestry and agriculture.

Effort has to be made to collect and preserve information on local names; chiefly in the areas inhabited by aboriginal tribes. Once this information is lost in the gigantic waves of rehabilitation and industrialisation, it may become impossible to avail these aspects of approach to many cultural problems or to get insight into several unknown facts about plants and their names.

Faithful field notes on vernacular plant names by field workers in agriculture, archeology, anthropology, etc., can greatly help. There is a caution signal, however. The recorder has to be selective. It is generally useful to ask more than one local person in the field party. Confirmation from different informers of the same region will make information more precise.

One is likely to meet the 'know-all' type among the informers, who may never like to show his ignorance. Such informer will lose no time in coining up a new name by permutation of the habit, habitat and colour, etc. of the plant. A tree (*Vriksha*) standing on a rocky (*Pashan*) plateau may be called *Pashan-vriksha*. But, when the same tree is incidentally found little later in the day in a marshy place or near water, it is interesting to observe the 'know-all' informer, who has, by then, forgotten the name *Pashan-vriksha* given by him. He looks at the plant, displays a blank appearance, looks at water where the tree is standing, makes a sly face and blurts out *Jal-vriksh* (*Jal* : water). One and same climber when growing over a *Nim* tree may be called *Nimlata*, and when on *Am*, *Amlata*.

A few instances of such mix-up can caution an intelligent recorder. With some discretion and experience, one is likely to collect useful information on local names, and through these names on a variety of uses of these plants.

It will thus be seen that the local names have a case for consideration. It is felt that the purpose for which the local names are given, i.e. an easy means of referring to plants of the region, is duly fulfilled by them, and local names are a useful and necessary agency for proper exploitation of the indigenous plant wealth.

#### **Summary :**

People know plants of their region for either their uses (as food, fodder, timber, medicine, etc.) or their injurious effects (like poisons, weeds, etc.) Useful as well as harmful plants which are required to be constantly referred by people are given vernacular names.

These names are often based on some salient feature of the plant, such as its shape, colour, habitat, uses, etc. Some examples of vernacular names as used in tribal areas of Madhya Pradesh are given with illustrations.

The chief merits and demerits of vernacular plant names are briefly discussed. Local names can not be used for scientific accounts of plants as they lack uniformity and consistency. But they render a useful service as a means of reference by local people.

Carefully recorded local plant names can yield useful information on botany, indigenous medicine, anthropology and certain other branches of knowledge.

#### **Acknowledgement :**

I am grateful to the Director, Botanical Survey of India for granting me facilities for visiting the tribal areas of Madhya Pradesh, and to the local officers there for help in field work.

#### **Literature Cited :**

Kirtikar, K. R. and Basu, B. D. *Indian Medicinal Plants* 4 vols., 1935.

Santapau, H. *Curcuma pseudomontana* Grah. *J. Bomb. nat. Hist. Soc.*, **45** : 618-623, 1945.

# ON THE STRUCTURE AND MECHANISM OF THE GENITALIA OF *AULACOPHORA*

By

R. S. SAINI

*Department of Zoology, University of Sagar, Sagar*

[Received on September 29, 1960]

## Introduction :

A large number of authors have worked on the reproductive and copulatory organs of coleoptera among whom the works of Verhoeff (1893), Sharp and Muir (1912), Sharp (1918), Muir (1918, 1919 and (1919a), Crampton (1921 and 1923), Tanner, (1927), Metcalfe (1932), deserve special mention.

Among the members of the family Chrysomelidae, Revnay (1928) has described the genitalia of the female *Leptinotarsa decemlineata* and Khatib (1946) has described the reproductive organs in female *Galerucella birmanica* and Mukerji and Chatterji (1951) of family Bruchidae and Saini (1954) has described the reproductive organs of *Aulacophora foveicollis* Luc. but as far as the author is aware the mechanism and musculature of the male and female genitalia of beetles has not been studied in detail and so in this paper an attempt has been made to describe the male and female genitalia and the musculature in *Aulacophora*.

## Material and technique :

Specimens of *A. foveicollis* were collected locally from Sagar and its neighbourhood, from different cultivated plants of the family cucurbitaceae. For dissections, both fresh as well as preserved material was employed. The insects were killed in KCN bottles and dissected under a binocular dissecting microscope. A beam of light was focussed on the specimens from Nachet or Reichert microscope lamp using 6 volts, 5 ampere bulbs. In order to preserve insects for the purpose of dissection, Kahle's fluid was found most satisfactory and specimens preserved in this fluid were best dissected in water and not in alcohol. For a study of the musculature preserved specimens were found to be more satisfactory than fresh specimens. The musculature was also studied by the help of microtome sections.

## Observation :

The male genitalia (Fig. 1) are enclosed in a genital pocket which is situated at the posterior end of the abdomen between the eighth tergum and the seventh sternum. The eighth tergite is much smaller than the seventh tergite and fits into the convexity on the ventral side of the seventh tergite. The basal margin of the eighth tergite is produced into two large processes to which large muscles arising from the semi-circular plate of the seventh sternite are attached. Beyond the eighth tergite, the ninth tergite is small and membranous and the membranous wall of the genital chamber is attached to it dorsally and to the seventh sternite ventrally as in the male the sternum of eighth and ninth segments are absent. The intromittent organ is enclosed in this genital chamber and can be protruded out of it.

The intromittent organ is developed as median, tubular, ectodermal evagination of the genital pocket and the terminal section of the ductus ejaculatoris is enclosed in it. According to Sharp and Muir (1912), in Coleoptera the intromittent organ is divisible into a proximal portion known as the tegmen or phallobase, and a distal portion called the median lobe; the latter encloses a sac like portion called the internal sac. The ductus ejaculatoris opens at the base of the internal sac and from this place arises an armature which often takes the form of an elongated transfer apparatus. In *Aulacophora*, the tegmen is greatly reduced and is represented by a narrow collar situated round the median lobe. In the present account, the term aedeagus will, therefore, be used as synonymous with the median lobe of Sharp and Muir. The internal sac has also been termed the endophallus (Snodgrass, 1935). Thus, in *Aulacophora* the tegmen or phallobase is membranous and not highly sclerotised as in many beetles. This membranous connection, however, is sclerotised at two places in the form of incomplete rings surrounding the median lobe or aedeagus. Each takes the form of a pair of sclerotised bars which are joined to each other ventrally, but are connected above the median lobe by membrane only; the ventral portion is further produced into an obliquely directed process. The sclerotised part of the anterior ring has been called the tegmen and its ventral process the strut of the tegmen; the sclerotised portion of the posterior ring has been called the spiculum and its ventral process the strut of the spiculum (Sharp and Muir, 1912). The median lobe or aedeagus is a highly sclerotised, tubular structure. In the normal condition, the internal sac is folded inside the median lobe and is membranous. The internal sac, however, is provided with scattered clumps of small, chitinous teeth along its inner surface, so that when it is everted, the teeth project out from its external surface. The number of these teeth is variable in the different species, being greatest in *A. foveicollis*, Luc. fewer in *A. atripennis*, Fab. and least in *A. cincta* Fab. The transfer apparatus is in the form of a highly sclerotised, elongated, somewhat curved, gutter-shaped structure and the male genital aperture opens at its base. Distally, the transfer apparatus is provided with a large number of strong recurved spines.

During copulation, the internal sac is everted out, so that the aedeagus (median lobe), the internal sac, and the transfer apparatus, together form an efficient intromittent organ for the transference of the sperms into the body of the female.

#### *Muscles of the Male Genitalia* (Fig. 1, 2, A. B. C.) :

In the male, the aedeagus and the internal sac are provided with several muscles which help in the protraction and retraction of the male genitalia.

The muscles which help in the protraction and retraction of the genital pocket and the aedeagus, are as follows :

##### (i) The tergo-sternal protractor muscle (m. 132) :

These are represented by numerous muscle fibres which arise from the posterior face of the semi-circular plate of the seventh sternite, and are inserted on the large lateral processes at the basal margin of the eighth tergite. The contraction of these powerful muscles pushes backwards the entire semi-circular plate and thus the genital pocket (along with the contained male genitalia) which is situated behind it, is pushed out. The structures revert back into their normal position, when the muscles relax.

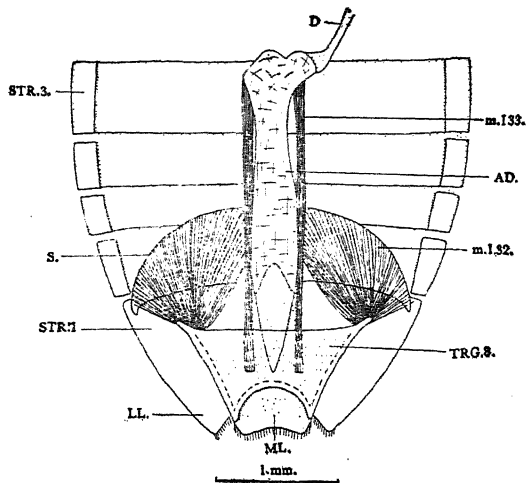


Fig. 1

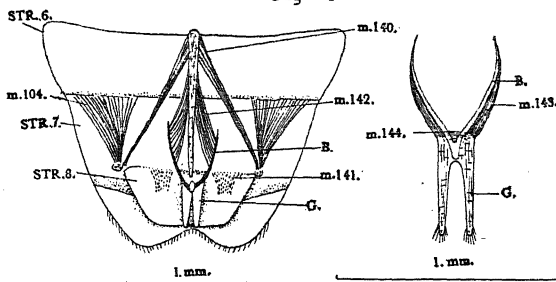


Fig. 3A

Fig. 3B

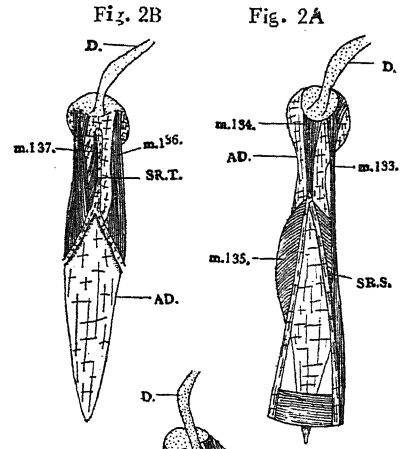


Fig. 2B

Fig. 2A

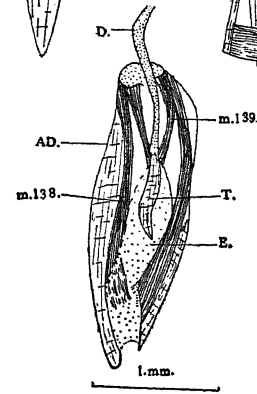


Fig. 2C

- Fig. 1. *A. foveicollis* Luc. Diagram of dissection from the dorsal side, showing some of the muscles associated with the male reproductive organs.
- Fig. 2A. *A. foveicollis*. Diagram of the aedeagus and tegmen from ventral side showing the muscles passing from the tegmen to the aedeagus.
- Fig. 2B. *A. foveicollis*. Diagram of the aedeagus and the spiculum as seen from the ventral side showing the muscles passing from the spiculum to the aedeagus.
- Fig. 2C. *A. foveicollis*. Diagram of the male genitalia with the aedeagus—opened out to show the muscles—associated with the internal sac and the transfer apparatus.
- Fig. 3A. *A. foveicollis*. Diagram showing the muscles associated with the female genitalia.
- Fig. 3B. *A. foveicollis*. Diagram showing the muscles associated with the genital palps and baculi.

#### KEY TO LETTERING

A.D. Aedeagus ; B. Baculi ; E. Endophallus ; G. Genital palp ; L.L. Lateral lobe ; M.L. Median lobe ; m. 104. Vento-lateral abdominal muscle of the 7th segment ; m. 132. Tergo-sternal protractor muscle of the male genitalia ; m. 133. Sternal protractor muscle of aedeagus ; m. 134. Spicular protractor muscle of aedeagus ; m. 135. Spicular retractor muscle of aedeagus ; m. 136. Tegminal protractor muscle of aedeagus ; m. 137. Tegminal retractor muscle of aedeagus ; m. 138. Protractor muscle of internal sac. m. 139. Retractor muscle of internal sac ; m. 140. Sterno-spicular protractor muscle of female genitalia ; m. 141. Sterno-genital protractor muscle of female genitalia ; m. 142. Spiculo-baculi retractor muscle of female genitalia ; m. 143. Paculo-palpal abductor muscle of female genitalia ; m. 144. Palpal abductor muscle of female genitalia ; S. Semicircular plate ; S.R.S. Strut of spiculum ; S.R.T. Strut of tegmen ; S.T.R. Sternite ; T. Transfer apparatus ; T.R.G. Tergum.

(ii) The sternal protractor muscles of the aedeagus (m. 133) :

These are represented by a pair of elongated muscle bands which arise from the sides of the median lobe of the seventh sternite and are inserted on the proximal end of the aedeagus. The contraction of these muscles pushes out the aedeagus beyond the genital pocket.

(iii) Spicular protractor muscle of the aedeagus (m. 134) :

This is represented by a single median muscle which arises from the anterior apex of the spiculum and is inserted on the anterior ventral edge of the aedeagus. Its contraction also helps to push out the aedeagus.

(iv) Spicular retractor muscles of the aedeagus (m. 135) :

These are represented by a pair of well developed muscles which arise from the sides of the spiculum near the apex and are inserted further back on the aedeagus. Their contraction retracts the aedeagus.

(v) Tegminal protractor muscles of the aedeagus (m. 136) :

These are represented by a pair of muscles which arise from the tegmen and are inserted forwards on the proximal end of the aedeagus. Their contraction helps in pushing out the aedeagus.

(iv) Tegminal retractor muscle of the aedeagus (m. 137) :

This is represented by a median ventral muscle which arises from the apex of the strut of the tegmen and is inserted backwards on the aedeagus. Its contraction helps in the retraction of the aedeagus.

The muscles of the internal sac are represented by the following:

(1) Protractor muscles of the internal sac (m. 138) :

These are represented by numerous muscle fibres which ensheath the posterior portion of the internal sac and then extend backwards as a pair of muscle bands which are inserted on the anterior end of the aedeagus. Their contraction everts the posterior portion of the internal sac and thus the inner surface of the internal sac which is armed with the teeth as well as the transfer apparatus, is exposed.

(2) Retractor muscles of the internal sac (m. 139) :

These are represented by a pair of small muscles which arise from the place where the transfer apparatus is attached to the wall of the internal sac and are inserted on the anterior end of the aedeagus. Its contraction pulls back the internal sac and the transfer apparatus back in the aedeagus.

*Female genitalia.* The female genitalia (Fig. 3) are enclosed in a genital pocket which is also situated at the hinder end of the abdomen below the eighth tergum and the ninth sternum which is without a semicircular plate. The eighth and ninth sternites play an important part in the formation of the female genitalia. In the female, the sternum of the eighth segment is present in the form of a small plate which is notched behind; its posterior and lateral margins are sclerotised but the anterior portion is membranous. From the posterior sclerotised portion of this sternum a long slender rod-like process or spiculum projects forward in the mid-ventral line and extends almost up to the anterior border of the sternite of the sixth segment. The sternum of the ninth segment seems to be absent, but at the hinder end of the segment there is a small, median, strongly sclerotised piece which is prolonged anteriorly into a pair of long, slender, horn-like processes

known as the baculi (Tanner, 1927), which are embedded in the ventro-lateral regions of the female genital chamber. A pair of small, unsegmented, elongated processes known as the genital palps project backwards from either side of the median basal piece referred above and the vulva is situated between these palps. The opposing mesial surfaces of the two genital palps are specially thickened and grooved, so that when apposed together, they enclose between them a narrow channel through which the eggs pass to the outside. The genital palps, the baculi and the median piece from which the latter arise, represent the elements of the female genitalia.

#### *Muscles of the Female Genitalia* (Fig. 3 A and B).

In the female, the genital chamber is provided with three sets of muscles, two of which act as protractor and one as retractor. These are as follows :

##### (i) The sterno-spicular protractor muscles (m. 140) :

These are represented by a pair of elongated muscles which arise from the anterior apex of the spiculum of the eighth sternite and are inserted on the lateral regions of the seventh sternite near its middle, close to the insertion of the ventro-lateral muscles of the seventh sternite. Their contraction causes the spiculum and the eighth sternite to be pushed backwards, and thus the genital chamber, which is attached to the eighth sternite, is protruded out to a limited extent.

##### (ii) The sterno-genital protractor muscles (m. 141) :

These are represented by a pair of small muscles which arise from the posterior folded portion of the seventh sternite and running forward on the ventral surface of the genital chamber. Their contraction also causes the genital chamber to be protruded out and act as auxiliaries to the preceding muscles.

##### (iii) The spiculo-baculi retractor muscles (m. 142) :

These are represented by a pair of muscle bands which arise from the anterior end of the spiculum and are inserted on the inner borders of the baculi. Their contraction serves to pull back the female genital chamber, which has been protracted out by the action of the two preceding muscles.

The genital palps are also provided with two sets of muscles which are as follows :

##### (i) Baculo-palpal abductor muscles (m. 143) :

These are represented by a pair of muscles, one on each side. Each muscle is attached to the base of the genital palp on the external side, and running forward is inserted on the sides of the corresponding baculi. Their contraction causes the two genital palps to diverge away from one another and thus allow easier passage to the eggs on their way out.

##### (ii) Palpal abductor muscles (m. 144) :

These are represented by muscle bands which pass from one genital palp to the other near their base. Their contraction causes the two genital palps to be apposed to one another more closely.

#### **Discussion :**

Both *Aulacophora* and *Galerucella* belong to the same sub-family Galerucinae, but they differ considerably from one another in the structure of the male genitalia. These differences are as follows :

- (i) In *Aulacophora foveicollis*, two Y-shaped, lightly chitinised pieces are present in connection with the male genitalia of which the anterior one is known



as the tegmen and its strut, while the posterior one is known as the spiculum gastrale and its strut. In *Galerucella birmanica*, only one such Y-shaped piece is present. Sharp and Muir, in their account of the male genitalia in two members of the sub-family Galerucinae, *Diabrotica*, and *Galerucella*, have described this Y-shaped piece as the tegmen and its strut; Khatib, on the other hand, states that 'there is not the slightest development of tegmen in this beetle', and has identified the Y-shaped sclerite shown in his fig. 33, as the spiculum gastrale and its strut. Whatever, may be the case in *Galerucella*, it is important to note that in *Aulacophora*, the condition of the male genitalia as represented by two Y-shaped pieces, is more typical, while it is more reduced in *Galerucella*. In the present author's view, that Y-shaped piece of *Galerucella* corresponds to the spiculum gastrale and its strut of *Aulacophora* and that it has been correctly named as such by Khatib. In *G. birmanica*, there is a pair of median hook like processes projecting downwards from the basal part of the aedeagus and called the 'median struts' by Khatib (1946), but no such structures are present in *Aulacophora*.

- (ii) In *Aulacophora*, there is a well developed, protrusible transfer apparatus, which is armed with spines and setae. This normally lies within the internal sac and the ductus ejaculatoris open at its base. A transfer apparatus is absent in *Galerucella*, and in it the ductus ejaculatoris communicates directly with the internal sac.
- (iii) The internal sac is armed with clumps of small chitinous teeth (which are most numerous in *A. foveicollis*, less in *A. atripennis* and least in *A. cincta*). When the internal sac is everted during copulation, these teeth project out and hold the intromittent organ in position within the female genital chamber. In *Galerucella*, the internal sac is devoid of any such armatures.

Sharp and Muir, (1912) have described a typical aedeagus consisting of two parts, i.e., a tegmen or a phallobase and a median lobe. As suggested by Metcalfe (1932), the present author thinks it advisable to restrict the term aedeagus to the median lobe only, as in many insects like *Aulacophora*, *Galerucella*, etc., well developed and chitinised tegmen is absent and in these species the term aedeagus is indifferently used for the intromittent organ which corresponds only to the median lobe of other insects.

The muscles of the male genitalia have not been described in *Galerucella* or any other member of the sub-family Galerucinae. Among the Phytophaga, Muir (1919) has described the muscles of the male genitalia in *Rhynchophorus* and *Strangalia*, but as the structure of the male genitalia in these insects and *Aulacophora* differ considerably, it is impossible to make a comparison of the muscles in the two cases. One point, however, needs special mention. Muir (1919) has observed that the protrusion of the internal sac "is always brought about by blood pressure, and it is highly probable that the different types have followed certain lines of evolution to accommodate the different development of the sacs, and allow for their functional mechanism." In *Aulacophora*, however, there are definite protractor muscles (m. 138) which by their contraction evert the internal sac. The retractor muscles of the internal sac (m. 139), however, are essentially similar in the *Aulacophora* and *Rhynchophorus*, though they differ in details due to the difference in the structure of the aedeagus and internal sac.

In *Aulacophora*, the female genitalia are represented by a pair of small, elongated, unjointed, closely approximated, genital palps, which are grooved mesially.

The genital palps are attached to a small, median, strongly chitinised, basal sclerite, which gives off anteriorly, a pair of curved rod-like processes or baculi, as described by Tanner (1927) in Chrysomelidae. In *G. birmanica*, also the female genitalia are represented by a pair of small, unjointed genital palps, but in this insect, they are widely separated off from one another and are not grooved mesially. Further, in *G. birmanica*, the two genital palps are borne on chitinous plates (and not on a single, median sclerite as in *Aulacophora*) and they have no anteriorly directed processes or baculi. The baculi as named by Tanner (1927) in the beetles described by him and also met with in *Aulacophora*, correspond to the 'chitinous rods' associated with the genital chamber by Revnay (1923) in *Leptinotarsa* and the anterior prolongations of the basal sclerites or 'valvifers' of the genital palps, as described by Crampton (1929).

(ix) In *Aulacophora*, the two genital palps act as an ovipositor and their two opposing mesial grooves serve as a tube through which the eggs are passed to the outside during oviposition. In *G. birmanica*, the genital palps, which are widely separated and do not act as ovipositors.

(\*) The muscles associated with the female genitalia have not been described by Khatib in *G. birmanica*, or as far as the author is aware, in any member of the family Chrysomelidae. Thus, a discussion of the musculature associated with the female genitalia in *Aulacophora* is not feasible. One point, however, may be noted and it is, that Tanner (1927) has not shown any muscle attached to the baculi, but in *Aulacophora*, two sets of muscles are attached to the baculi, the spiculo-baculi retractor muscles (m. 142) which by their contraction pull back the genital chamber, and baculo-palpal abductor muscles (m. 143), the contraction of which causes the two genital palps to somewhat diverge away from one another and thus allow easier passage of the eggs to the outside.

#### Summary :

1. In *Aulacophora* the 8th sternite is absent in the male but it is present in the female.
2. The 9th tergite is membranous in the male but it is chitinised in the female.
3. In the male genitalia two Y-shaped chitinous structures are present which corresponds to the tegmen and its strut and the spiculum gastrale and its strut respectively.

The intromittent organ consists of an aedeagus and internal sac, and a transfer apparatus armed with spines. The internal sac is armed with clumps of small chitinous teeth.

There are six muscles which helps in protraction and retraction of the male genitalia. Muscles are also provided in the internal sac

4. In the female there are a pair of small unjointed, closely approximated genital palps which are grooved mesially. They are attached to a small median chitinised plate which is produced anteriorly into a pair of baculi. The female genital chamber is provided with three sets of muscles.

The genital palps are also provided with two sets of muscles.

#### Acknowledgment :

The author is very much thankful to Prof. D. S. Srivastava for valuable guidance and suggestions.

## References :

- Crampton, G. C. Correction of a Statement concerning the Terminal Abdominal Structures of Male Insects. *The Canadian Entomologist*, **53** : 72-90, 1921.
- The terminal abdominal structures of female insects compared throughout the orders in accordance with phylogeny. *J. N. Y. Ent. Soc.*, **37** : 453-496, 1929.
- Imms, A. D. A General Text-Book of Entomology. London, 1957.
- Khatib, S. M. H. The external morphology of *G. birmanica*. *Proc. Ind. Acad. Sci.*, B, **23** : 1-38, 1946.
- Metcalfe, M. E. The structure and development of the reproductive system in Coleoptera, with notes on its homologies. *Quart. J. Micr. Sci.*, **75** : 49-129, 1932.
- Muir, F. Notes on the Ontogeny and Morphology of male genital tube in Coleoptera. *Trans. Ent. Soc. London*, **1** : Part II : 223-230, 1918.
- The male abdominal segments and aedeagus of *Habrocerus capillaricornis* Grav. *Trans. Ent. Soc. London*, **77** : 398-402, 1919.
- On the mechanism of the male genital tube in Coleoptera. *Trans. Ent. Soc. London*, **77** : 404-412, 1919a.
- Mukerji S. and Chatterjee, S. N. Morphology of the genital structures of some of the Bruchidae of India and Ceylon and their taxonomic importance. *Ind. J. of Ent.*, **13** Part I : 1-28, 1951.
- Revnay, E. External Morphology of the Colorado Potato beetle (*Leptinotarsa decemlineata*, Say). *J. N. Y. Ent. Soc.*, **36** : 125-137, 1928.
- Saini, R. S. Studies on the reproductive organs of *Aulacophora*. Saugar University Jour. **1** (3) : 81-89, 1954.
- Sharp, D. and Muir, F. The comparative anatomy of the male genital tube in Coleoptera. *Trans. Ent. Soc., London*, **12** : 477-642, 1912.
- Snodgrass, R. E. Principles of Insect Morphology. New York. 1935.
- Tanner, V. M. A preliminary study of the genitalia of female Coleoptera *Trans. Amer. Ent. Soc., Phila.*, **53** : 5-50, 1927.
- \*Verhoeff, K. W. Vergleichende Untersuchungen über die Abdominal segmente und die Copulationsorgane bei den Coleopteren. *Deutsch Entom. Zeits.*, 113-170, 1893.

---

\* Not seen in original.

# EFFECT OF SOME PHYSICAL FACTORS ON THE GENUS *ONYCHIURUS* (COLLEMBOLA)

By

D. K. CHOUDHURI

Entomology-laboratory, University Science College, Kalyani University, Kalyani  
(Nadia Dt., West Bengal)

[Received on December 13, 1962]

## Introduction :

Of the various environmental factors influencing the life-history and bionomics of the Collembolan microfauna, soil moisture and humidity have been critically studied by Davies (1928), Ripper (1930), Davidson (1932), Strebel (1932), MacLagan (1932) and Agrell (1941). Observations are however lacking in *Onychiurus*, a fairly large group of soil-dwelling forms of some economic importance. The present paper records the results of work on the effects of drought and humidity under laboratory conditions on *O. fimatus*, *O. parthenogeneticus* and *O. imperfectus*, extracted by means of a modified Tullgren funnel from soil-samples, from England and reared in the laboratory. Throughout this paper the data for the three species are given in the following order : *O. fimatus*, *O. parthenogeneticus* and *O. imperfectus*.

## (A) Desiccation on the development of eggs :

In *Sminthurus viridis* Davidson (1932) has shown that when the soil-moisture falls below 7% for a long time, there is a marked decrease in the viability of eggs. In my experiments on *O. fimatus* an attempt was made to investigate the resistance of eggs of different ages to conditions of drought, which must occasionally prevail in their natural habitat.

*Expt.* The eggs were dried by exposure on a dry black filter paper in a petri dish at 50% relative humidity. After desiccation, which varied in duration, all the eggs were transferred to 100% R.H. There were 9 sets, each consisting of 7 series of 4 batches of eggs. The eggs in the different sets were laid on successive days and one set of eggs was placed at 24°C on each of 9 successive days. On each subsequent successive day one series of eggs from each set was dried as described above, so that 7 series of eggs in each set had 1-7 days desiccation.

The largest number of viable eggs is contained in the sets V-VII (i.e. 4-6 days old eggs). Not a single egg of any set hatched during the period when the eggs were subjected to drying. In sets before and after those mentioned above there is a reduction in the survival of eggs, especially in those which had already undergone less than 36% or more than 55% of their development prior to being exposed to desiccation. Moreover the comparison of the figures in Table 1 with those for the eggs not subjected to desiccation (Choudhuri, 1963) indicates that the prevalence of drought conditions may temporarily arrest the development. It is, however, resumed as soon as the eggs are returned to favourable conditions of moisture.

## (B) Humidity :

### (a) Survival of individuals of various ages.

*Expt.* In order to expose the individuals to atmospheres, the humidity of which was controlled at a known level, a series of 1000 ml. flat-bottomed flasks contained silica gel (gave an effective dry atmosphere), mixtures of acid and water

graded to provide relative humidities of 10, 20, 30, 50, 70, 85, 95 and plain water for 100% R. H. The humidities were checked with a Gregory hygrometer and the specific gravities of the acid/water mixtures were periodically determined with hydrometers. The flasks containing the empty glass tubes (to take the insects) were stoppered with rubber bungs and left at a constant temperature of 24°C for at least 24 hours before the introduction of the Collembola. Five insects were enclosed by muslin seals in micro-tubes, which had previously been exposed to the required humidity and each tube was returned to the appropriate flask, in which it was suspended by cotton thread. The humidifying solutions were replaced from time to time. The introductions of the batches of insects were performed as rapidly as was practicable so as to avoid abrupt humidity variations by atmospheric contaminations. Five replicates were carried out for the individuals of each stage. The results of this experiment are summarised in table 2.

TABLE 1  
Showing the survival of eggs of various ages under desiccation

Set I						
Series of 4 egg batches	No. of eggs	Days of desiccation at 24°C	Hatching started days after the transfer of eggs	No. of eggs hatched	% of no hatch	Days of incubation prior to being desiccated
A	47	7	—	0	100.0	0
B	48	6	—	0	100.0	0
C	45	5	10	1	97.8	0
D	49	4	10	5	90.0	0
E	47	3	10	9	80.8	0
F	51	2	10	15	70.6	0
G	56	1	10	18	60.8	0
Set II						
A	45	7	—	0	100.0	1
B	44	6	—	0	100.0	1
C	51	5	9	3	94.1	1
D	50	4	9	7	86.0	1
E	45	3	9	11	75.5	1
F	48	2	9	16	66.6	1
G	46	1	9	19	58.7	1
Set III						
A	46	7	—	0	100.0	2
B	48	6	—	0	100.0	2
C	45	5	8	4	91.0	2
D	49	4	8	10	80.0	2
E	44	3	8	14	68.0	2
F	45	2	8	17	62.2	2
G	45	1	8	21	53.3	2

## Set IV

Series of 4 egg batches	No. of eggs	Days of desiccation at 24°C	Hatching started days after the transfer of eggs	No. of eggs hatched	% of no hatch	Days of incuba- tion prior to being desiccated
A	44	7	—	0	100.0	3
B	43	6	7	2	95.3	3
C	50	5	7	6	88.0	3
D	51	4	7	12	76.5	3
E	47	3	7	16	66.0	3
F	46	2	7	19	60.0	3
G	45	1	7	22	51.0	3

## Set V

A	47	7	—	0	100.0	4
B	47	6	6	4	91.5	4
C	44	5	6	7	84.1	4
D	48	4	6	13	73.0	4
E	47	3	6	19	64.0	4
F	51	2	6	23	55.0	4
G	45	1	6	24	47.0	4

## Set VI

A	47	7	5	2	95.7	5
B	48	6	5	7	85.4	5
C	48	5	5	12	75.0	5
D	47	4	5	17	64.0	5
E	44	3	5	19	56.8	5
F	46	2	5	24	48.0	5
G	45	1	5	27	40.0	5

## Set VII

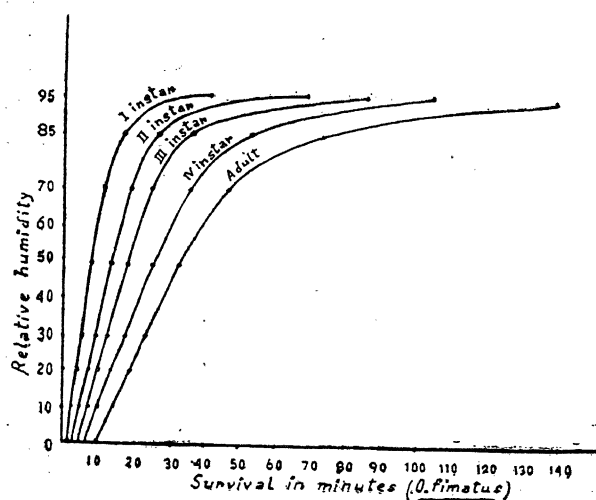
A	45	7	—	0	100.0	6
B	50	6	4	5	90.0	6
C	44	5	4	9	80.0	6
D	47	4	4	15	68.0	6
E	46	3	4	18	60.8	6
F	52	2	4	25	52.0	6
G	45	1	4	26	42.2	6

# Set VIII

Series of 4 egg batches	No. of eggs	Days of desiccation at 24°C	Hatching started days after the transfer of eggs	No. of egg hatched	% of no hatch	Days of incubation prior to being desiccated
A	44	7	—	0	100.0	7
B	46	6	—	0	100.0	7
C	50	5	3	1	98.0	7
D	48	4	3	1	95.8	7
E	45	3	3	6	86.6	7
F	41	2	3	10	75.6	7
G	50	1	3	17	66.1	7

# Set IX

A	45	7	—	0	100.0	8
B	48	6	—	0	100.0	8
C	47	5	—	0	100.0	8
D	46	4	—	0	100.0	8
E	50	3	2	2	96.0	8
F	48	2	2	7	85.4	8
G	45	1	2	11	75.5	8



The maximum resistance is found in the adults and their mean length of life increased from 8.9, 3.6 and 6.6 minutes at 0% R.H. to 43.7, 32.3 and 35.0 days respectively. This increase did not become so marked until the relative humidity approached 90%. At 85% R.H. the adults of the three species survived for only 75.0, 27.4 and 60.0 minutes respectively. The first instar of the three species was the most sensitive and died within 40.7, 26.5 and 30.1 minutes respectively at 95% R.H. The survival time of the different stages at the relative humidities from 0-95% has been graphically tested in *O. fimatus* (Text fig. 1). The trend of curve obtained may be taken as an indicator of the reaction of other related species. At low humidities the individuals of all ages found to be moving actively up and down the vertical sides of the tubes housing them.

TABLE 2

Showing mean length of life in minutes at 0-95% and  
in days at 100% R.H.

*O. fimatus.*

Relative humidity									Stages
0%	10%	20%	30%	50%	70%	85%	95%	100%	
1.5	2.2	4.0	5.6	7.7	11.1	16.5	40.7	—	I instar
2.7	5.0	7.5	10.0	13.9	18.5	27.5	68.9	—	II instar
3.9	6.7	9.6	12.2	16.9	24.5	36.9	86.0	—	III instar
6.0	10.0	14.9	18.8	25.4	35.0	54.4	104.8	—	IV instar
8.9	14.5	19.0	23.8	33.0	46.2	75.0	139.2	43.7	Adult

*O. parthenogeneticus*

1.0	1.5	2.2	3.0	4.7	6.0	9.1	26.5	—	I instar
1.5	2.7	3.8	4.8	7.0	9.5	13.4	36.1	—	II instar
2.2	3.5	4.6	6.0	8.6	11.4	17.0	42.7	—	III instar
3.0	4.6	6.9	8.5	11.4	15.5	23.0	54.0	—	IV instar
3.6	5.5	7.6	9.7	13.5	18.3	27.4	62.8	32.3	Adult

*O. imperfectus.*

1.1	2.0	3.5	5.0	7.0	10.5	15.1	30.1	—	I instar
2.0	4.0	6.5	9.0	12.0	16.5	24.9	47.1	—	II instar
3.0	5.8	8.5	12.2	16.0	22.1	35.0	59.9	—	III instar
4.0	7.7	11.7	15.3	19.7	27.5	44.0	74.1	—	IV instar
6.6	10.4	15.0	19.0	24.9	39.1	60.1	95.6	35.0	Adult

(b) *Survival of eggs.*

25 eggs of each species immediately after they were laid, were exposed to the different relative humidities, mentioned in the previous experiment, for 20 days. It was found that the eggs of the three species exposed to 100% R.H. developed normally and hatched, but no hatching occurred even at 95% R.H.



At further batch of 25 eggs of each of the three species, allowed to develop for 6 days at 100% R.H. at 24°C, were then exposed to the different relative humidities for 15 days. It was found that hatching occurred only in the eggs exposed to 100% R.H. All the eggs in other relative humidities collapsed.

(c) *Excess of moisture.*

(i) *Newly laid eggs submerged under water* : Two series of 25 eggs of *O. fimatus* and *O. parthenogeneticus* were kept under water in glass containers 4.5 cm in height and 5.0 cm in diameter at 24°C and at 20°C in the case of *O. imperfectus*. Of these 39, 40 and 35 respectively of the different species mentioned here hatched, but the period of incubation was more prolonged than that of the eggs incubated at favourable conditions (Choudhuri, 1963). Some individuals of the I instar died soon after hatching, but others survived for 5-6 days and without moulting.

(ii) *Partially developed eggs submerged under water* : In 2 series of 50 eggs of each species, the eggs of both *O. fimatus* and *O. parthenogeneticus* have an incubation period of 6 days at 24°C, but in *O. imperfectus* 9 days at 20°C prior to being submerged under water. Similar glass containers were placed at the same temperature as above (Table 3).

TABLE 3  
Showing development of eggs under water

a = Early eggs  
b = Advanced eggs

	No. of eggs	Date of laying	No. of eggs hatched	Developmental period in days	% Mortality	Temp.	Species
(a)	50	14.2.57	39	12	22.0	24°C	<i>O. fimatus</i>
(b)	50	1.3.57	50	11	0.0		
(a)	50	2.3.57	40	13	20.0	24°C	<i>O. parthenogeneticus</i>
(b)	50	3.3.57	50	12	0.0		
(a)	50	3.8.57	35	19	30.0	20°C	<i>O. imperfectus</i>
(b)	50	4.8.57	50	18	0.0		

There is 100% hatching in all species and the incubation period at this temperature was almost the same as that of the eggs incubated at saturated moisture condition (Choudhuri, 1963).

**Discussion :**

Holdaway (1927) has shown the extraordinary resistance of the undeveloped eggs of *Sminthurus viridis* to dry conditions. Similar observation through more extensive and better-planned experiments by Davidson (1932) shows that partially developed eggs (i.e. eggs which have undergone about 35-50% of their development prior to being exposed to dry conditions) are more resistant than either the early eggs or the more advanced eggs. The observations presented

here show that the eggs of *Onychiurus* behave almost similarly and likewise, there is noticed a decrease in the number of the viable eggs with the increase in the incubation prior to the eggs being desiccated. Ripper (1930), while working on *Hyogastrura*, came across a similar "resistant stage" in development. From the evidence available it seems that probably the presence of such phenomenon i.e. "resistant stage" is widespread and common in other soil-dwelling fauna of Collembola.

Increased activity at low humidities was first reported by Rudolph<sup>1</sup> (1923-1925), who, working on mosquitoes, found that upto 94-95% R.H. activity is accelerated, but on approaching the saturation point the activity shows a sharp drop. A similar result is seen in *Onychiurus* and this may be due to an avoidance of the low humidities. Comparison of survival times of the three species, here, at various humidities with those of other species [Davies (1928), Ripper (1930), Strebel (1932) and Agrell (1941)] has shown that *Onychiurus* is in no way atypical in this respect, falling well within the wide range of tolerance for the Collembola as a whole.

There are many early reports of the lethal effects of low relative humidities on insect eggs, such as those of Janisch (1930) on *Prodenia*, Peterson (1920) on aphids and Bodenheimer (1930) on *Schistocerca gregaria*. The last named author found that earlier stages of eggs are more susceptible to low humidity than the advanced eggs. The results of the present investigation show that in *Onychiurus* early and advanced eggs are equally susceptible to low humidities and they require almost saturation humidity for their optimal development. This finding is consistent with that of Davies (1928), who found that even in 98% R.H. eggs do not develop. It is not unexpected that any soil-dwelling form like *Onychiurus* should need an atmosphere at or near saturation. Investigations of Thamdrup (1939) show that if the water content of soil exceeds 20% by weight, the atmosphere is essentially saturated. Above all, it is also known that air in between soil-particles may maintain a saturated atmosphere by means of "bound water" which does not dry up so easily.

The supersaturated condition of the soil prevails when heavy showers cause the pores of low-lying parts to be completely filled with water, which sometimes takes a long time to sink into the lower layers. The present work indicates that under such conditions the susceptibility of the early eggs is greater than that of the advanced eggs. A relatively greater mortality and a longer developmental period are characteristic of the early eggs submerged under water. Davidson (1932) also found a similar detrimental effect of the super saturated condition on the eggs of *Siminthurus viridis*.

#### Summary :

- (1) Resistance of eggs to desiccation varies with the age of the eggs, both early and advanced eggs are more susceptible than others.
- (2) Adults are more resistant to low humidity than the juveniles *O. parthenogeneticus* is more susceptible than other two species. The eggs fail to develop even at 95% R.H.

#### Acknowledgments :

Grateful acknowledgment is made to Prof. O. W. Richards, F.R.S., Imperial College of Science & Technology, London, for facilities for work in his laboratory. Thanks are also due to Mr. H. E. Goto of the same institution for valuable discussions.

---

1. For detailed literature see Uvarov B. P. (1931).

## References :

- Agrell, L. Zur Ökologie der Collembolen. *Öpusc. Ent. Suppl.*, **3** : 1-236, 1941.
- Bodenheimer, F. S. and Kleim, H. Z. Über die Temperaturabhängigkeit von Insekten. ii. Die Abhängigkeit der Aktivität bei der Ernteameise *Messor semirufus*, E. André von Temperatur und anderen Faktoren. *Z. Vergl. Physiol.*, **11** : 345-385, 1930.
- Choudhuri, D. K. Temperature and its effect on the three species of the genus *Onychiurus* : Collembola. *Proc. Zool. Soc.*, **16** (1) : 97-117.
- Davidson, J. On the viability of the eggs of *Sminthurus viridis* L. (Collembola) *Aust. J. exp. Biol. med. Sci.*, **10** : 65-88, 1932.
- Davies, W. M. The effect of variation in relative humidity on certain species of Collembola. *Brit. J. exp. Biol.*, **6** : 79-86, 1928.
- Holdaway, F. G. The bionomics of *Sminthurus viridis* Linn. of the South Australian lucerne flea. *Commonw. Australia, Counc. sci. & indust. Res. Pamphl.*, **4** : 1-23, 1927.
- Janisch, E. Experimentelle Untersuchungen über die Wirkung der Umweltfaktoren auf Insekten. I Die Massenvermehrung der Baumwolleneule *Prodenia littorlis* in Ägypten. *Z. Morph. Okol. Tiere*, **17** : 339-416, 1930.
- MacLagan, D. S. An ecological study of the "lucerne flea" (*Sminthurus viridis* L.) I-II. *Bull. ent. Res.*, **23** : 101-145, 151-190, 1932.
- Paterson, A. Some studies on the influence of environmental factors on the hatching of the eggs of *Aphis avenae*, Fab., *Aphis pomi*, De Geer, and *Aphis sorbi*, Kalt. *J. econ. Ent.*, **10** : 556-560, 1920.
- Ripper, W. Champignon-Springschwänze. Biologie und Bekämpfung von *Hypogastrura manubrialis* Tullb. *Z. angew. Ent.*, **16** : 546-584, 1930.
- Strebel, O. Beiträge zur Biologie, Ökologie und Physiologie einheimischer Collembolen. *Z. Morph. Okol. Tiere*, **25** : 31-153, 1932.
- \*Thamdrup, H. M. and Nielsen, E. T. Bioklimatische Beiblätter, **6** (4) : 180-184, 1939.

---

\*Not seen in original.

# THE UTILIZATION OF RAFFINOSE BY SOME PATHOGENIC SPECIES OF *PHYLLOSTICTA*

By

K. S. BILGRAMI

*Department of Botany, University of Allahabad, India*

[Received on August 8, 1963]

Trisaccharide raffinose is associated with sugar complex of many green plants. Enzyme  $\beta$ -fructosidase is usually functional during the earlier stages of its utilization by fungi with the result that melibiose and fructose are the only two component sugars to be traced in the medium. Rapid utilization of fructose and accumulation of melibiose in the medium supplied with raffinose has been reported for *Ceratocystis fimbriata* and *Thielaviopsis basicola* (Wilson and Lilly, 1958) as well as *Pestalotia banksiana* and *P. citri* (Tandon and Bilgrami, 1958). The above findings clearly show that these fungi attack raffinose at one linkage only since the breakdown products of the other linkage i.e. sucrose and galactose were not spotted in the culture solution.

In the present communication the behaviour of eight pathogenic species of *Phyllosticta* towards raffinose as well as its various hydrolytic products has been presented.

## Materials and Methods :

The test isolates were the same as used by the author earlier (Bilgrami, 1963). The basal medium consisted of glucose 10 g,  $\text{KNO}_3$  3.5 g,  $\text{KH}_2\text{PO}_4$  1.75 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.75 g, thiamine  $100\mu$  g, biotin  $10\mu$  g, trace elements so as to furnish 0.1 mg each of iron and zinc and 0.05 mg of manganese per litre, and double distilled water one litre. Raffinose and its derivatives were substituted singly for glucose of the basal medium. Twenty-five ml of steam sterilized liquid nutrient contained in 150 ml Erlenmeyer flasks served as substrate. The flasks were inoculated with different pathogens and incubated at  $25^\circ\text{C}$ . Three incubation periods at the intervals of five days were used. After completion of the incubation time the fungal colonies were filtered and the accomplished dry weights were used for judging the comparative value of different sources. The method followed by Tandon and Bilgrami (1958) was used for chromatographic analysis of the medium.

## Experimental Results :

The dry weight yield of eight species of *Phyllosticta* on raffinose, its various hydrolytic products and their combinations is presented in Table 1.

TABLE I

Showing the dry weight (in mg.) of various species of *Phyllosticta* attained on raffinose and its hydrolytic products

Sugars	Days of incubation	Names of the species							
		<i>P. baulniae</i>	<i>P. butee</i>	<i>P. flavidula</i>	<i>P. dracaenae</i>	<i>P. dardanoi</i>	<i>P. kigeliae</i>	<i>P. glaucispora</i>	<i>P. pandanicola</i>
Raffinose	5	42	30	34	33	58	28	22	40
	10	69	48	59	60	99	48	30	79
	15	89	65	80	72	130	64	41	94
$\left\{ \begin{array}{l} \frac{1}{3} \text{ fructose} + \\ \frac{2}{3} \text{ melibiose} \end{array} \right.$	5	40	33	36	33	40	29	14	42
	10	76	62	77	74	97	62	30	72
	15	100	86	93	103	149	83	38	94
$\left\{ \begin{array}{l} \frac{1}{3} \text{ fructose} + \\ \frac{1}{3} \text{ glucose} + \\ \frac{1}{3} \text{ galactose} \end{array} \right.$	5	50	30	30	69	59	31	40	49
	10	110	54	56	120	112	58	72	92
	15	138	75	80	154	140	70	96	130
$\left\{ \begin{array}{l} \frac{1}{2} \text{ melibiose} + \\ \frac{1}{2} \text{ sucrose} \end{array} \right.$	5	29	30	34	42	40	22	15	30
	10	80	72	82	96	103	58	42	87
	15	120	96	111	128	154	70	56	117
Glucose	5	52	38	65	76	56	38	27	69
	10	90	58	118	122	93	56	53	100
	15	113	70	148	162	121	82	74	129
Fructose	5	48	29	59	54	48	32	22	49
	10	79	52	100	92	91	57	40	91
	15	102	64	122	130	107	61	51	121
Galactose	5	20	39	26	76	22	23	26	26
	10	27	66	62	112	80	42	49	69
	15	106	76	83	146	117	57	64	98
Melibiose	5	19	23	10	12	46	20	6	25
	10	44	38	30	39	96	40	16	54
	15	64	58	47	53	123	56	25	76

Results from Table 1 distinctly establish that there was considerable variation in response of different isolates towards raffinose and its component sugars.

The results of daily chromatographic analysis of the medium containing raffinose solution are recorded in Table 2.

TABLE 2

Showing the days of the persistence of various sugars produced in raffinose medium during the growth of different species of *Phyllosticta*

	Names of the species							
	<i>P. bauhiniæ</i>	<i>P. buteæ</i>	<i>P. flavidula</i>	<i>P. dracaenæ</i>	<i>P. dardanoi</i>	<i>P. kigeliae</i>	<i>P. glaucispora</i>	<i>P. pandanicola</i>
Raffinose	1-6	1-5	1-6	1-8	1-4	1-6	1-11	1-6
Fructose	2-8	2-7	2-8	2-9	2-6	2-7	3-7	2-6
Melibiose	2-15	2-13	2-15	2-25	2-9	2-18	2-25	2-21
Glucose	5-7	6,9-10	11,14	6,11,14	4-10	4,7, 10-12	13,16	4-7
Galactose	5-8	8-12,14	8-11, 14-17	11-13,18	3-12	4-9, 13-16	13-16	4-12
Oligosaccharide I	-	6-7	4	5-7	4-7	-	-	4-6

Results from Table 2 indicate that raffinose was broken at  $\alpha$  linkage by all the present species of *Phyllosticta* with the result that melibiose and fructose were the only two hydrolytic products to be formed initially. All the species consumed fructose much earlier than the melibiose portion. They hydrolysed the melibiose part with varying efficiency. Its hydrolytic products i.e., glucose and galactose were detected in a regular series only on those media which were utilized by *P. bauhiniæ*, *P. dardanoi* and *P. pandanicola*. In all other cases the component sugars were detected only infrequently. An oligosaccharide ( $R_f$  0.08) was synthesized by *P. buteæ*, *P. flavidula*, *P. dracaenæ*, *P. dardanoi* and *P. pandanicola*. Persistence of the oligosaccharide was usually short and the period varied with the species.

### Discussion :

It is evident from Table 1 that five species viz. *P. bauhiniæ*, *P. buteæ*, *P. dracaenæ*, *P. dardanoi* and *P. kigeliae* accomplished good growth on raffinose. Its unsatisfactory utilization by other species is obviously due to slow hydrolysis of melibiose moiety which remains accumulated in the medium almost upto 25 days in most of the cases. The results also reveal that growth attainment of these species was slightly higher on raffinose than on melibiose. A comparative study of dry weight and chromatographic data indicates that even those species which hydrolysed melibiose fraction comparatively slowly gave better growth on raffinose after first five days as compared to corresponding growth on melibiose alone. Major fraction of this growth achievement is evidently at the expense of fructose component which is readily available in the culture medium from the second or third day and is consumed latest by the 9th day. Even those species which easily cause the cleaving of melibiose do not appear to attack this disaccharide from the second day as in most cases its hydrolytic products were spotted from the 5th or the 6th day, which shows that perhaps the fructose unit contributes towards the major fraction of growth attained during first five days even for those fungi which hydrolyse melibiose with

comparatively faster rate. This conclusion is also supported by the fact that *P. flavidula* and *P. glaucispora* which did not utilize raffinose very efficiently obviously due to slow *melibiase* activity accomplished comparatively much better growth on raffinose than on melibiose after first five days. The results manifest that the present species hydrolyse raffinose with greater ease and rapidity than melibiose and the transformation of raffinose to melibiose and fructose was not a limiting factor for its slower availability to certain species because both these products were obtainable in the culture medium from the second or third day.

Species of *Phyllosticta* expressed great versatility towards the individual sources as well as the combinations of various mono- and disaccharides obtained from the hydrolysis of raffinose. *P. bauhiniae*, *P. glaucispora* and *P. pandanicola* gave maximum preference to a mixture of three monosaccharides. A mixture of two disaccharides *i.e.*, sucrose and melibiose was slightly better than other combinations or individual sources for *P. buteae* and *P. dardanoi*. Chromatographic analysis of the medium showed that these two species converted the entire sucrose from this mixture to fructose and glucose within 24 hours. The glucose fraction consumed within first three days so that only fructose and melibiose were left on the fourth day. Glucose was again traced in the medium along with galactose from the 5th or 6th day indicating the hydrolysis of melibiose. *P. flavidula* and *P. dracaenae* attained best growth on glucose as the sole carbon source. A mixture of three mono-saccharides was almost similar to glucose for these two species. *P. kigeliae* had best hyphal output on mixture of fructose and melibiose or glucose alone. The dry weight results also manifested that final growth on two substances may be alike but the rate of growth may not be similar. Several Moniliales including *Chalara quercina* (Beckman *et al.*, 1953); *Fusarium oxysporum f. nicotianae* (Wolf, 1955), *Fusarium coeruleum* (Agarwal, 1955) and *Cercosporina ricinella* (Sudhir Chandra, 1961) show as good response for raffinose as for glucose. A number of fungi (Lilly and Barnett, 1953; Durairaj, 1956 and Hall, 1959) are however, unable to utilize raffinose which is definitely due to *raffinase* inactively in those forms. Chromatographic data further manifested that most of the species which hydrolysed the melibiose fraction faster synthesized an oligosaccharide (*Rf* 0.08) on the raffinose medium. This oligosaccharide was very transitional and persisted for few days only.

#### Summary :

The response of eight pathogenic species of *Phyllosticta* towards the trisaccharide raffinose was studied in detail. *P. bauhiniae*, *P. buteae*, *P. dracaenae*, *P. dardanoi* and *P. kigeliae* expressed quite favourable inclination for this substance. Regular chromatographic analysis of the culture solution showed that in the initial stages only melibiose and fructose were produced in the medium. Unsatisfactory utilization of raffinose by the remaining three species appears to be due to slow *melibiase* activity in those forms. Species of *Phyllosticta* manifested varying degree of choice for different components of this trisaccharide. *P. bauhiniae*, *P. glaucispora* and *P. pandanicola* showed maximum inclination for a mixture of three monosaccharides *viz.*, glucose, galactose and fructose while *P. buteae* and *P. dardanoi* gave preference to a combination of disaccharides *i.e.*, melibiose and sucrose. *P. flavidula* and *P. dracaenae* accomplished best growth on glucose as the sole carbon source while best hyphal density of *P. kigeliae* was recorded on a mixture of fructose and melibiose.

#### Acknowledgement :

The author is very grateful to Prof. R. N. Tandon for providing the facilities for this work,

## References :

- Agarwal, G. P. D.Phil. Thesis, University of Allahabad, 1955.
- Beckman, C. H., Kuntz, J. E. and Riker, A. J. The growth of oak wilt fungus with vitamins, carbon and nitrogen sources. *Phytopathology* **43** : 441-447, 1953.
- Bilgrami, K. S. Amino acid composition of some pathogenic species of *Phyllosticta*. *Flora* **153** : 49-53, 1963.
- Durai Raj, V. Growth of *Colletotrichum capsici* in pure culture. *Jour. Indian bot. Soc.* **35** : 409-413, 1956.
- Hall, A. M. The culture of *Phytophthora infestans* in culture. *Trans. Brit. Mycol. Soc.* **33** : 132-141, 1951.
- Lilly, V. G. and Barnett, H. L. The utilization of sugars by fungi. *West Va. Univ. Agric. Expt. Stat. Bull.* 362 T. 5-58, 1953.
- Tandon, R. N. and Bilgrami, K. S. The utilization and synthesis of oligosaccharides by two species of *Pestalotia*. *Proc. Natl. Inst. Sci.* **24** : 118-124, 1958.
- Wilson, E. M. and Lilly, V. G. The utilization of oligosaccharides by some species of *Ceratocystis*. *Mycologia*. **50** : 376-389, 1958.
- Wolf, F. T. Nutrition and metabolism of the tobacco wilt *Fusarium*. *Bull. Torrey. Bot. Club.* **82** : 343-354, 1955.



# INFLUENCE OF DIFFERENT SOIL TEMPERATURES ON THE INCIDENCE OF *FUSARIUM* WILT OF GRAM (*CICER ARIETINUM* L.)

By

S. K. CHAUHAN

*Botany Department, Agra College, Agra*

[Received on September 24, 1962]

L. R. Jones and his collaborators presented a monographic account of their work on the effect of soil temperature on plant diseases in 1926, Garrett (1944) has given a tabulated list of diseases favoured by high and low soil temperatures. The wilt of pea caused by *Fusarium oxysporum* f. *pisi* progressed most rapidly at 27°–30°C with nutrient solution (Schroeder and Walker 1942). The present paper deals with the study of the influence of different soil temperatures on the development of wilt of gram (*Cicer arietinum* Linn.) caused by *Fusarium orthoceras* App. and Wr. var. *ciceri* Padwick.

## Method and Material :

Glazed aluminium tumblers (10"×5") filled with garden soil were used to raise the seedlings. These pots were fixed in temperature tanks designed after those of Wisconsin and maintained at 15°, 20°, 25°, 30° and 35°C ( $\pm 1^\circ\text{C}$ ). The inoculum was raised on corn-meal-sand medium and equal quantities of the same were mixed thoroughly with the soil in the experimental pots. Five days after infestation of the soil three seeds of gram (variety T 87), were sown in each pot. For each temperature treatment ten inoculated and three uninoculated (Control) pots were kept. Optimum conditions were provided for the disease development as investigated by the author (1959). Mortality figures were recorded at regular intervals of four days and the final figures of total mortality have been analysed statistically and critical difference calculated. The pathogen was reisolated from the wilted plants to make sure the cause of their death. However, in control sets all the plants remained healthy throughout the experiment.

## Results :

The observations recorded are summarised below :

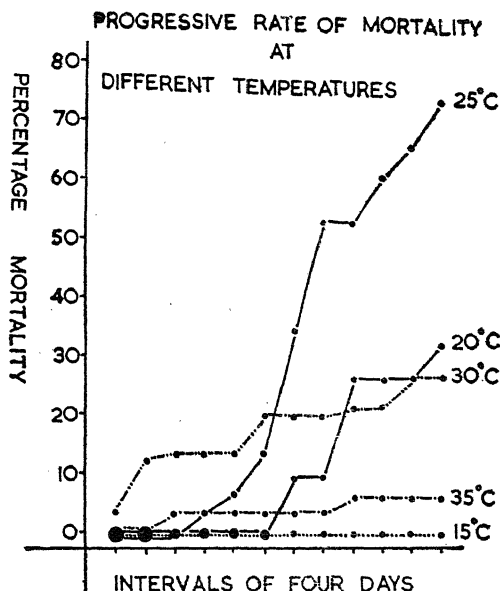
TABLE I  
Showing percentage mortality  
(Mean of ten pots with three seeds in each pot)

Different temperatures in °C				
15	20	25	30	35
0.0	32.0	72.7	26.5	5.7
$\pm 0.0$	$\pm 1.2$	$\pm 3.1$	$\pm 1.1$	$\pm 0.2$

Critical difference at 5% level 16.9

The results in table I indicate that the percentage mortality is maximum at 25°C and it decreases with increase or decrease in soil temperature. At 15°C the disease did not develop at all.

The progressive rate of percentage mortality at different temperatures has also been observed. There is no progress in 15°C; at 20°C the progress in mortality rate is after one month's time and reaches to a maximum of 32 percent; at 25°C also the appearance of the disease is late but in this case reaches upto 73%; at 30°C the disease has got a very study increase but becomes constant at lower level of 27 percent, but at 35°C there is very slow increase and does not go beyond 6%. All these trends of disease development are clear in the graph presented.



#### Conclusion :

25°C is the optimum temperature for the disease under investigation. If the soil temperature is reduced or increased the incidence of the disease also becomes less. This has also been indicated by Sadasivan (1961). This temperature is also suitable for the growth of the parasite (Chauhan, 1963) and for toxin production (Chauhan 1961) and for PP enzyme secretion (Gupta 1962). It is, therefore, suggested that by the change of date of sowing, which alter the soil temperature considerably, the disease can be controlled effectively. This was also shown in field trials by Padwick and Bhagwagar (1943).

#### Summary :

Wilt of gram (*Cicer arietinum* L.) caused by *Fusarium orthoceras* App. and Wr. var. *ciceri* Padwick has been studied in relation to different soil temperatures. Five temperatures viz. 15°, 20°, 25°, 30° and 35°C were maintained in the temperature tanks designed after those of Wisconsin. Final mortality figures were analysed statistically and critical difference calculated. It has been observed that the percentage disease incidence was maximum at 25°C and it reduced considerably when the soil temperature was increased or decreased. At lower temperature (15°C) there was no disease development.

### Acknowledgements:

The author is grateful to Prof. S. Sinha for valuable suggestions and to Scientific Research Committee, U. P., for the contingent grant.

### References :

- Chauhan, S. K. Studies on wilt of gram and crop-loss estimates. Ph.D. Thesis, Agra University, 1959.
- Toxin production by *Fusarium orthoceras* var. *ciceri* causing wilt of gram. *Proc. Nat. Acad. Sci. (India)* Sec. B. **31** (3) : 341-348, 1961.
- Growth and reproduction of *Fusarium orthoceras* var. *ciceri* causing wilt of gram (*Cicer arietinum* L.) at different temperatures. *Proc. 50th Indian Sci. Cong.*, Part III, 347, 1963.
- Garrett, S. D. *The Root Disease Fungi*. Chronica Botanica. Waltham, Mass. U. S. A., 1944.
- Gupta, M. N. Studies in the pathogenicity of certain parasitic fungi with special reference to their pectic enzyme system. Ph.D. Thesis, Agra University, 1962.
- Jones, L. R., Johnson, J. and Dikson, J. G. Wisconsin studies upon the relation of soil temperature to plant disease. *Res. Bull. Wis. Agri. Exp. Sta.*, 71, 1926.
- Padwick, G. W. and Bhagwagar, R. P. Wilt of gram in relation to date of sowing. *Indian Jour. Agri. Sci.*, **8** (3) : 287-290, 1943.
- Sadasivan, T. S. Physiology of wilt diseases. *Ann. Rev. Plant Physiology*, **12** : 449-468, 1961.
- Schroeder, W. T. and Walker, J. C. Influence of control environment and nutrition on the resistance of garden peas to *Fusarium* wilt. *Jour. Agri. Res.* **65** : 221-248, 1942.

## MORPHOLOGY OF THE THORAX OF ARAEOPIDAE (HOMOPTERA, FULGOROIDEA)

By

R. D. SAKSENA and A. N. T. JOSEPH

Department of Zoology, Balwant Rajput College, Agra

[Received on December 10, 1962]

The three species of Araeopidae (Delphacidae) included in the present study belong to two tribes, viz., *Liburnia pallescens* (Distant), *Delphacodes propinqua* (Fieber)—tribe Delphacini—and *Purohita cervina* Distant—tribe Tropidocephalini, of the subfamily Araeopinae (Delphacinae) chosen especially to establish the relationship between these two tribes on the basis of their thorax.

There hardly exists any detailed account of the morphology of the thorax of araeopids except by Mathur and Joseph (1961). But workers like Myers (1928), Qadir and Aziz (1950) and Akbar (1957) have studied the morphology of the thorax of various bugs and others like Crampton (1909), Snodgrass (1909, 1935) and Martin (1916) etc. have dealt with the morphology of generalised insects.

### The cervix :

In araeopids the cervix or neck is concealed beneath the overlapping anterior region of the pronotum dorsally as well as laterally. The anterior pair of cervical sclerites is obsolete in the insects under observation, this reduction can be correlated with the limited movements of the head. The posterior pair (Fig. 2, Cp) appears as small, sclerotised projections at the anterior margin of episterna of prothorax and touch the posterior margin of occipital foramen.

### The prothorax :

Prothorax (Figs. 1, 2 and 3) is an independent segment, quite broad dorsally and narrow ventrally whose tergum, pleura and sternum are fused together. The tergopleural lines of fusion are distinct beneath the pronotum, but there are no traces of sternopleural lines.

The pronotum is collar-shaped and posteriorly roofs over the dorsal and lateral regions of mesothorax. Its anterior margin bears a thin ridge with which is attached the neck membrane. In *Purohita cervina* the lateral ends of pronotum are directed laterally whereas in the other two species they are facing ventrally. The pronotum is devoid of sutures unlike that of pterothoracic nota. It has three mediolongitudinal carinae or keels running from the anterior to the posterior margin. The keels are stout, highly developed and reach the hind margin in *Purohita cervina* but in *Liburnia pallescens* and *Delphacodes propinqua* the carinae are comparatively thin and only the median carina extends to the hind margin. The lateral carinae run divergently posterad and disappear before reaching the hind border. The lateral carinae gradually narrow down posteriorly in *Liburnia pallescens* and *Delphacodes propinqua*, but in *Purohita cervina* they are almost of uniform thickness throughout their length.

The propleura and the prosternum are fused together to form the propectus occupying the ventral and ventrolateral regions of prothorax. The pleuron is

reduced and for a greater part is hidden beneath the laterally expanded region of the pronotum. The pleural region is membranised, especially that situated beneath the laterally projecting tergum. Along with this membranisation, the pleural suture becomes obsolete, but the pleural ridge is distinct which extends from the pleural articular process anteriorly and thus demarcating internally the pleural into the episternum and epimeron. The episternum bears at the anterior margin the posterior cervical apodeme and continues ventrally as a narrow sclerite, the precoxale, anterior to the coxal cavity to unite with the sternum. Similarly the epimeron joins with the sternum posterior to the coxal cavity by another narrow sclerite, the postcoxale. The leg is articulated medially to the pleural region by the pleural articular process. In addition to this, there is another anterolateral attachment by means of the trochantin, which is a small, distally tapering sclerite. It arises from the episternum where the two supracoxal arches meet or near the pleural ridge and curves round anteroventrally to articulate with the coxal rim. Internally the pleural ridge gives out the pleural apophysis, which is directed ventrally to meet with a corresponding apophysis to form the thoracic furca (Fig. 4). The prothoracic furca is smaller than the mesothoracic furca, but is much larger in proportion to the size of prothorax. It is more laterally orientated in *Purohita cervina* than in *Liburnia pallescens* and *Delphacodes propinqua* and is of uniform thickness throughout its length. In *Liburnia pallescens* and *Delphacodes propinqua* it is swollen medially and directed internally. The first spiracle is located laterally in the intersegmental membrane between the first and second thoracic segments roofed over by the laterally expanding protergum.

The prosternum is a narrow transversely elongated plate extending between the pleura. It is divided into two by a faint transverse sternacostal suture, bearing internally the sternacosta and dividing the sternum into an anterior basisternum and a posterior sternellum. The sternal apophyses arise from the lateral sides of the sternacosta and extend dorsolaterally to unite with the pleural apophyses.

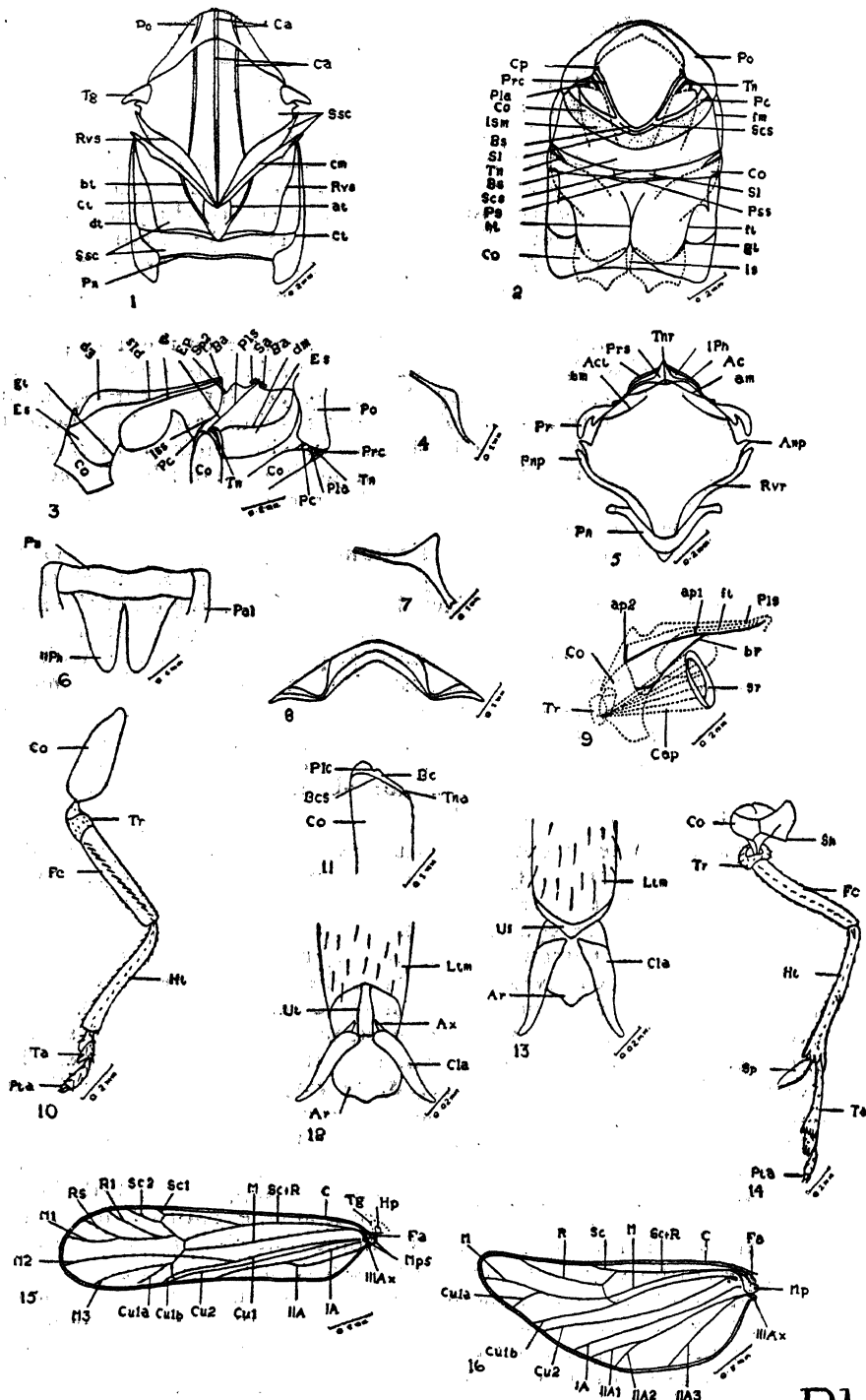
#### **The mesothorax :**

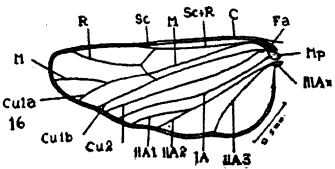
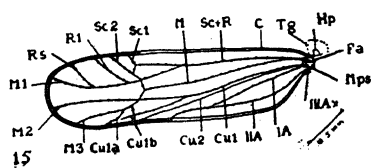
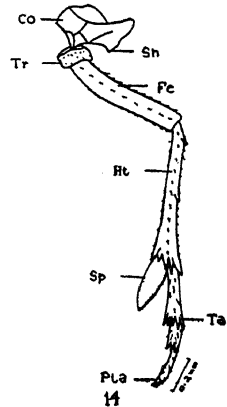
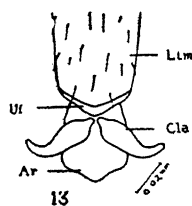
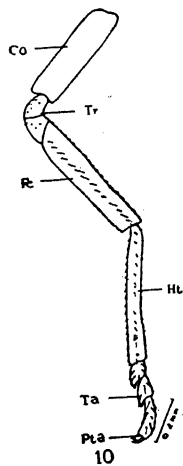
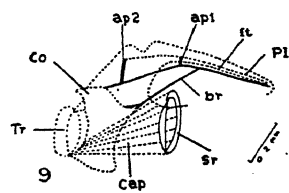
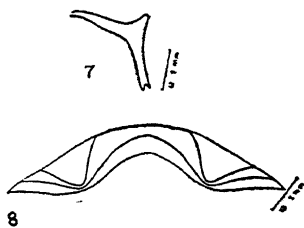
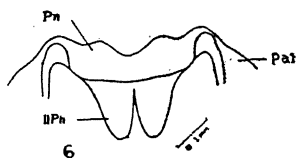
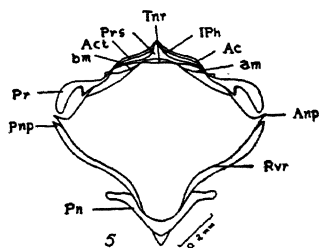
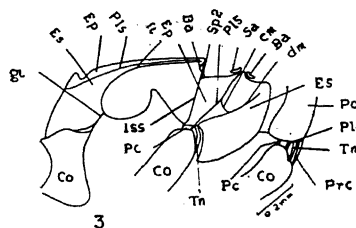
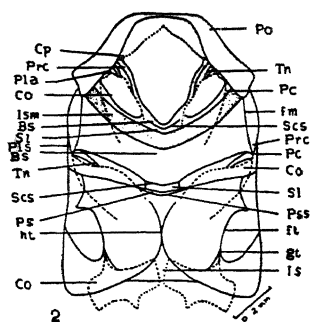
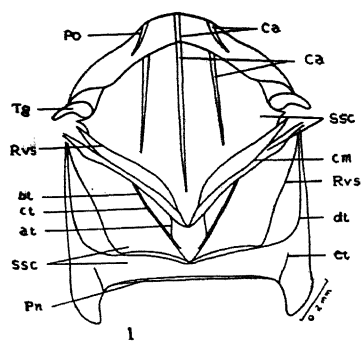
The mesothorax (Figs. 1, 2 and 3) constitutes the largest part of the thorax. There are distinct tergopleural sutures separating the tergum from the pleura, but the pleural and sternal regions are fused to form a mesopectus.

The mesonotum is a large arched plate pointed both anteriorly as well as posteriorly and telescoped anteriorly into the posteriorly projecting pronotum. It has three longitudinal carinae, one in the middle and the remaining two at the lateral sides and gradually narrowing from the proximal to the distal region. In *Liburnia pallescens* and *Purohita cervina* the carinae reach the hind border, in the former all the carinae and in the latter the median carina are faint posteriorly, whereas in *Delphacodes propinqua* they vanish before reaching the hind margin. The mesonotum can be primarily divided into an anterior large wing bearing alinotum and a posterior narrow postnotum. Towards the anterior end, the alinotum (Fig. 5) has a prescutal or transverse notal suture dividing the alinotum into an anterior narrow prescutum and a posterior large scutoscuteillum. Internally, the prescutal suture bears a faint transverse notal ridge (Tnr.) The prescutum continues laterally towards the episternum anterior to the wing base as the prealare (Pr.) The prealare has a small, posteriorly directed projection in all the three species under observation which is more pointed and spine-like in *Purohita cervina*. There is a faint submarginal antecostal suture, anterior to the transverse notal suture, which cuts off a narrow acrotergite (Act). Internally the suture bears the antecosta (Ac) which expands ventrally to form the first phragma

(IPh). The first phragma is thin, feebly developed and deeply notched in the middle. It is comparatively well developed in the males of *Liburnia pallescens* and *Delphacodes propinqua* whereas it is uniformly developed in both the sexes of *Purohita cervina*. Besides these, there are two sutures (am, bm) lying at the anterior region of alinotum, taking their origin slightly posterior to the transverse notal suture. Of these, anterior one (am) arises near the transverse scutal suture, runs anterolaterally, crosses the latter suture after some distance and joins with the corresponding one from the opposite side. The other suture (bm) lies a little posterior to it and runs in the same direction as that of (am); it joins with the suture (am) after some distance in *Delphacodes propinqua*; in the other two species they vanish before joining with the anterior suture. The scutoscutellar suture is absent in araeopids and as a result of which the scutal and the scutellar regions are merged together. Posteriorly the alinotum bears a reversed notal or pseudoscutoscutellar suture (Fig. 1, Rvs), the area included inside this suture is raised well above the two lateral areas. Internally the suture develops a corresponding feebly developed ridge. The lateral areas of the scutoscutellum are folded beneath the middle raised region and are V-shaped. Hence, in situ their median region is not visible. This folded area of the scutoscutellum is secondarily divided by a V-shaped suture (cm) running almost parallel to the pseudoscutoscutellar suture. The axillary cord of the tegmen is joined to the scutellum. The postnotum is in the form of a narrow plate fused with the alinotum, completely hidden beneath the scutoscutellum. Sometimes it is in a very reduced condition so much that it is mistaken as the secondarily divided V-shaped posteriormost region of the alinotum with which it is fused as in *Peregrinus maidis* (Mathur and Joseph, 1961). The postnotum is V-shaped and laterally continues as postalare to unite with the epimeron similar to that of prealare. The postnotum carries at its hind margin the second phragma (Fig. 6, IIPh), which consists of a pair of thin conical plates continuous basally in *Liburnia pallescens* and *Delphacodes propinqua*, while in *Purohita cervina* its separation into two plates is complete. In all the species under study it is thickly sclerotised at its basal region for about one third distance and gradually narrows distally to form a thin plate.

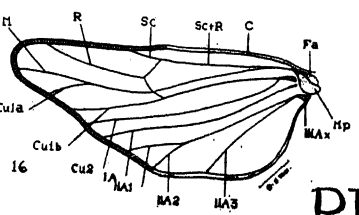
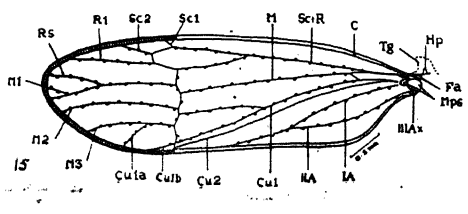
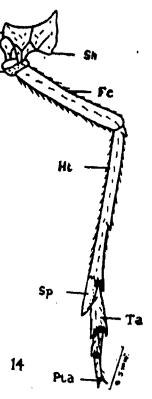
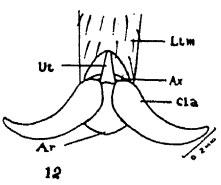
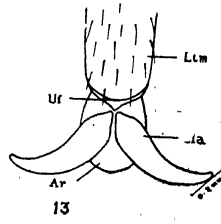
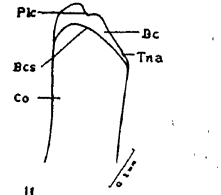
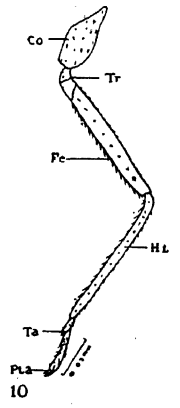
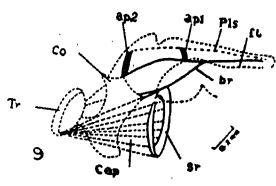
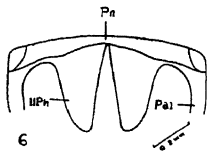
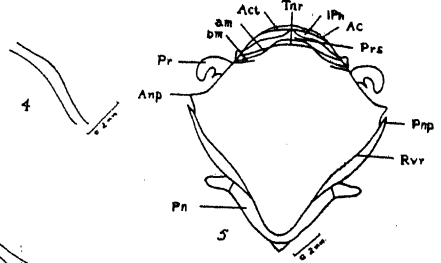
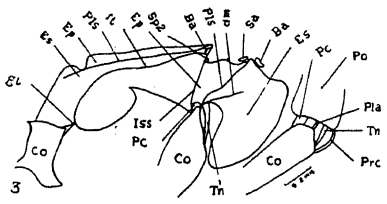
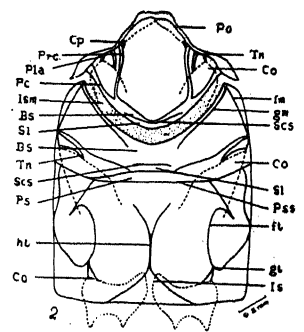
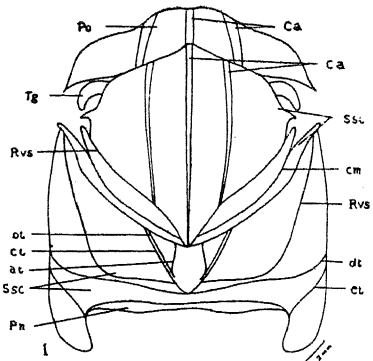
The pleural suture arises from the pleural articular process and extends upto the wing base. It takes an oblique course and in the middle it is slightly curved anteriorly. It divides the pleuron into an anterior large episternum and a posterior small epimeron. The episternum joins the sternum by a large precoxale, while the epimeron by a narrow postcoxale. The pleural suture internally develops the pleural ridge which gives out an apodeme to meet the corresponding apodeme coming from the sternum to form the mesothoracic furca (Fig. 7), similar to that of prothoracic furca. A little anterior to it, the pleural ridge gives rise to another small conical apodeme running parallel to the pleural apophysis. The mesopleuron develops secondary sutures (Fig. 3) in varying degree in different species studied. In *Liburnia pallescens* there is a suture (dm) originating a little posterior to the pleural suture and runs obliquely to join the anterior margin. In *Purohita cervina* this suture is short and does not extend more than half of the area of episternum. In *Delphacodes propinqua* the suture disappears a little before reaching the anterior boundary. Besides this, there is another suture (em) in this species starting from the pleural suture near its middle and joining to the suture (dm). Dorsal to the episternum, near the pleural suture, there is a small crescent-shaped basalare and similarly the epimeron has a small subalare. The second thoracic spiracle is located at the lateral side of the mesothorax at the junction between the tergum and the posterior corner of the epimeron. The trochantin is similar to that of the prothoracic segment in origin, attachment and shape, but has proportionately increased in size along with the large size of mesothorax.





Pl. II





Pl. III

## EXPLANATION OF PLATES

Plate I, *Liburnia pallescens*.

Plate II, *Delphacodes propinqua*.

Plate III, *Purohita cervina*.

- |  |  |
|--|--|
| Fig. 1. Dorsal view of thorax.                                     | Fig. 9. Lateral view of metapleuron showing the internal ridge and the apodeme projecting into the thorax from the trochanter. |
| Fig. 2. Ventral view of thorax.                                    | Fig. 10. Fore leg.   |
| Fig. 3. Lateral view of thorax.                                    | Fig. 11. Basal region of precoxa enlarged.   |
| Fig. 4. Prothoracic furca.   | Fig. 12. Ventral view of pretarsus.  |
| Fig. 5. Inner view of mesonotum.                                   | Fig. 13. Dorsal view of pretarsus.   |
| Fig. 6. Inner view of posinotum of mesothorax showing the phragma. | Fig. 14. Hind leg.   |
| Fig. 7. Mesothoracic furca.  | Fig. 15. Tegmen.   |
| Fig. 8. Third phragma.   | Fig. 16. Hind wing.  |

## ABBREVIATIONS

IA. first anal ; IIA. second anal ; IIA 1. first branch of second anal ; IIA 2. second branch of second anal ; IIA 3. third branch of second anal ; Ac. antecosta ; Act. acrotergite : amis secondary suture of mesothorax ; Anp. anterior notal wing process ; ap 1. first apophysis from the pleural suture to the pleural ridge ; ap 2. second apophysis from the pleural suture to the pleural ridge ; Ar. arolium ; at. secondary suture of metathorax ; Ax. auxilia ; IIIAx. third axillary ; Ba. basalar ; Bc. basicoxite ; Bcs. basicostal suture ; bm. secondary suture of mesothorax ; br. branch of metathoracic pleural ridge ; Bs. basisternum bt. secondary suture of metathorax ; C. costa ; Ca. carinae ; Cap. sclerotised projection extending from the hind trochanter into the thorax ; Cla. claw ; cm. secondary suture of mesothorax ; Co. coxa ; Cp. cervical apodeme ; ct. secondary suture of metathorax ; Cu 1. cubitus one ; Cu la. first branch of cubitus one ; Cu lb. second branch of cubitus one ; Cu 2. cubitus two ; dm. secondary suture of mesothorax ; dt. secondary suture of metathorax ; em. secondary suture of mesothorax ; Ep. epimeron ; Es. episternum ; et. secondary suture of metathorax ; Fa. fused first and second axillaries ; Fe. femur ; fm. secondary suture of mesothorax ; ft. secondary suture of metathorax ; gm. secondary suture of mesothorax ; gt. secondary suture of metathorax ; Hp. humeral plate ; ht. secondary suture of metathorax ; Ht. tibia ; Iss. intersternite ; Ism. intersegmental membrane between the pro and mesosternum ; Iss. intersegmental suture between the meso and metapleura ; Ltm. last tarsomere ; M. media ; M 1. first branch of media ; M 2. second branch of media ; M 3. third branch of media ; Mp median Plate ; Mps. median plates ; Pal. postalar ; Pc. postcoxale ; IPh. first phragma ; IIPh. second phragma ; Pla. pleural articulation ; Plc. concavity at the proximal margin of coxa for articulation of pleural process ; Pls. pleural suture ; Pn postnotum ; Pnp. posterior notal wing process ; Po. pronotum ; Pr. prealar ; Prc. precoxale ; Prs. prescutum ; Ps. pre-sternum ; Pss. pre-sternal suture ; Pta. pretarsus ; R. radius ; R 1. radial one ; Rs. radial sector ; Rvr. reversed notal ridge ; Rvs. reversed notal suture ; Sa. subalar ; Sc. sub-costa ; Sc 1. first branch of subcosta ; Sc 2. second branch of subcosta ; Sc+R. subcosta plus radius ; Scs. sternacostal suture ; Sh. spine at the distal margin of coxa of metathoracic leg ; Sl. sternellum ; Sp. spur ; Sp 2. second thoracic spiracle ; sr. sclerotised rim at the margin of the apodeme projecting into the metathorax from the hind trochanter ; Ssc. scutoscute lum ; Ta. tarsus ; Tg. tegula ; Tn. trochantin ; Tna. cavity at the proximal margin of coxa for the articulation of trochantin ; Tnr. transverse notal ridge ; Tr. trochanter ; Uf. unguifer ; Ut. unguitractor.

The eusternum (Fig. 2) is limited posteriorly by the intersegmental groove between the meso and metathorax. The sternacostal suture is a short, transverse suture in *Liburnia pallascens* and *Delphacodes propinqua*; it joins laterally with the posterior boundary of mesothorax to form a small rectangular sternellum and a large basisternum. In *Purohita cervina* the sternacostal suture is free from the posterior boundary of mesothorax and so the sternellum is not marked as a rectangular area. The sternacostal suture internally carries the sternacostal ridge whose lateral ends give out the sternal apophyses. In all the three species the mesopectus carries a pair of longitudinal sutures (fm), one on either side of the lateral margin extending from the coxal cavity near the pleural suture upto the anterior margin. In *Purohita cervina* the anterior margin of the basisternum has two submarginal sutures (gm) one on either side running parallel to the anterior margin of the eusternum and directed posteriorly near the middle.

### The metathorax :

The metathorax (Figs. 1, 2 and 3) is highly modified in Araeopids, especially the metapleuron, as a special leaping mechanism is developed in this segment. Curiously enough, it has no furca. The metathorax has distinct tergopleural lines to separate the notum from the pleura. Similar to that of pro and mesothorax, the pleura and sternum are united to form a metapectus.

The metanotum is V-shaped anteriorly and lies between the mesonotum and the first abdominal tergum. Laterally it is joined to the pleura by the tergopleural suture. It is divisible into a large wing bearing alinotum and a posterior narrow postnotum, situated between the corners of the posterolaterally projecting areas of the notum. Like that of the alinotum of mesothorax, the metanotum is devoid of a scutoscuteellar suture but is divided by a V-shaped pseudoscuteoscuteellar suture arising from the anterolateral corner of the alinotum. There are a number of secondary sutures displayed on the alinotum (Fig. 1). The dorsomedian area of alinotum bears a pair of sutures on either side and another one outer to these sutures forming a complete V-shaped suture. The innermost one (at) begins slightly lateral to the dorsomedian line, runs convergingly for a short distance and joins with another suture (bt). The latter suture begins lateral to the former and takes a similar course. The combined suture thus formed runs for a short distance and abruptly ends there. Immediately outer to the suture (bt) and parallel to it runs the third suture (ct) which meets its fellow from the opposite side to give a V-shaped suture. The area included in between the sutures (bt) and (ct) is raised as a ridge which continues beyond the combined (at) and (bt) to the tip of (ct). Outer to it and well apart from this runs the pseudoscuteoscuteellar suture. External to the latter suture runs another V-shaped suture (dt) in *Liburnia pallascens* and *Delphacodes propinqua* taking its origin near the pseudoscuteoscuteellar suture and posteriorly apposing or almost touching the pseudoscuteoscuteellar suture and then diverging before joining with its mate from the opposite side. In *Purohita cervina* it takes its origin from the posterior side of the tergopleural suture. From the region where the suture (dt) apposes or nearly apposes the pseudoscuteoscuteellar suture, the area of alinotum is raised as a ridge, this elevated area passes below the elevated area running along the suture (ct). At the posterolateral corners, one on either side, there is a small suture (et) directed anteriorly whose extension varies with the species. In *Liburnia pallascens* it curves to join the suture (dt); in *Delphacodes propinqua* it reaches only a little more than half of the area from its origin to the suture (dt); In *Purohita cervina* it diverges to join with the tergopleural suture. The postnotum is reduced and is fused to the posterior margin of alinotum. It carries internally the unpaired phragma (Fig. 8) whose sclerotisation is interesting. There is a distal membranous area surrounded by a thickly

sclerotised proximal area. At the lateral sides of the proximal area there are still thickly sclerotised conical areas. In *Liburnia pallescens* and *Purohita cervina* there is not much difference in the thickness of sclerotisation between the proximal and distal areas, whereas in *Delphacodes propinqua* the difference is quite conspicuous. The third phragmata are of uniform size in both the sexes of *Purohita cervina*, while in the males of tribe Delphacini their lateral thickly sclerotised areas extend internally as projections tapering distally.

The pleural suture is visible as extending from the wing process but stopping short of the coxal cavity. It divides the pleuron into a large episternum and a narrow epimeron. Lower to the pleural suture runs another suture (ft) parallel to it for considerable distance and then distally curves back to join the intersegmental line between the meso and metathorax (Fig. 3). From the posterior margin of the suture ((ft) extends another suture (gt) to the leg base which is bifurcated. At the anterolateral margin of episternum is located the basalare, but there is no subalare in the epimeron. The hind coxa is firmly united to the pleuron and as there is no movement the trochantin is also not developed. The pleural suture and the pleural ridge deserve special attention as they have undergone great modifications. The pleural ridge is in contact with the pleural suture at its base but is separated from the suture as it runs posteriorly. Towards the middle it is supported by an apodeme (ap1) and another one (ap2) at the distal region where the pleural suture terminates. As already mentioned the pleural suture stops before joining the coxal cavity but the ridge continues and is fused with the coxa; thus the division of the pleuron into the episternum and the epimeron is complete. From the middle of the pleural ridge arises a branch (br) which runs posteriorly and bifurcates into two before joining the coxal base. Externally towards the posterior side, the branch (br) is marked by a suture (gt). All these ridges form a firm skeletal frame work to support the pleuron so as to withstand the stresses and strains of the powerful leaping action of the hind legs.

The metasternum (Fig. 2) is comparatively well developed. The sternacostal suture is indistinct and the sternacosta is extending as a long, thin ridge, marked externally the entire length by a suture (ht). Hence it is difficult to demarcate the exact boundary between the basisternum and the sternellum. The sternal apophysis is not developed and there is no furca formation in the metathorax. Anteriorly the sternum has a transverse submarginal suture, the presternal suture, which cuts off a narrow presternum.

### **The legs :**

The coxo-trochanteral, the trochantero-femoral and the femoro-tibial joints are dicondylic, the remaining articulations are monocondylic. All the dicondylic articulations are horizontal except the trochantero-femoral which is vertical; the monocondylic articulations are dorsal. The fore and middle legs are similar in structure and equal in length, but the length of the individual regions are dissimilar. The hind leg is large and its various components have undergone modifications partly to fit in the role of leaping taken by it.

The coxa of the fore leg (Figs. 2, 3, and 10, Co) is elongated with a medially narrow region; the distal region is stouter than the proximal part. The proximal submargin of coxa (Fig. 11) is encircled by a faint basicostal suture which marks off the basicoxite from the coxa. The basicostal suture internally bears the basicosta, which strengthens the base of the coxa. The proximal margin of coxa is oblique with a distinct marginal rim. Proximally the marginal rim of coxa possesses a small concavity (Pic) at the dorsolateral side to which is articulated the pleural articular process. Anterior to this at the coxal rim there is another small concavity

(Tna) for the articulation of trochantin. Thus coxa has pleural as well as trochanteral articulations. Further, the coxal base is attached to the coxal socket by a thin membrane surrounding it—the coxal corium. The coxa is decorated with small scattered spines in *Purohita cervina*; but these are absent in *Liburnia pallescens* and *Delphacodes propinqua*. Distally, the coxa has a pair of articular surfaces to hinge with the trochanter by a dicondylic articulation. A thin conjunctival membrane joins the distal rim of coxa with the proximal rim of the segment following.

The trochanter (Fig. 10, Tr) is the smallest segment of the leg and is highly sclerotised. Its lumen is reduced by thick inflection of its wall near the middle. It externally bears small, scattered spines. The dorsal surface of the trochanter is short and concave while the ventral surface is longer and convex. From the trochanteral base arises a pair of thin apodemes, one from the dorsal and the other from the ventral side. They extend into the coxa, tapers distally and are articulated to the trochanteral base by membranes. They serve for the attachment of the trochanteral muscles. The distal rim of the trochanter is oblique. Its articulation with the femur is so fixed that the femur moves along with the trochanter and the conjunctival membrane between them is reduced.

The femur (Fig. 10, Fe) is almost equal in length to that of tibia in *Liburnia pallescens* and *Delphacodes propinqua*, while it is shorter than the tibia in *Purohita cervina*. Its proximal end is slightly stouter and it gradually narrows posteriorly. It is comparatively thinner in *Purohita cervina* than in the other two species. The femur has four or five longitudinal rows of spines of varying sizes. In *Liburnia pallescens* there are four rows of spines; dorsal, ventral, anterior and posterior. The anterior and posterior rows are large and of equal size, the dorsal row small and the ventral row is composed of minute spines. In the other two species, viz., *Delphacodes propinqua* and *Purohita cervina*, it has five longitudinal rows of spines; one dorsal, two ventrolateral, one anterior and the other posterior. In the former, the anterior, the posterior and the ventrolateral rows are comparatively well developed, the dorsal row being composed of small spines. In the latter, the anterolateral row is the largest, the posterolateral row larger, the dorsal row large, the anterior and posterior rows are small and basally apart. There are no apodemes attached to the femur as it has got no independent movement.

The tibia (Fig. 10, Ht) is generally the longest segment in araeopids. It is slightly bent proximally; it is broad distally in *Liburnia pallescens* and *Delphacodes propinqua*, while it is *vice versa* in *Purohita cervina*. It has five longitudinal rows of spines displayed in the same fashion as that of the preceding segment of *Delphacodes propinqua* and *Purohita cervina*. Its row of largest spines is much smaller than the row of the largest spines of the femur. Of these, the ventrolateral rows are larger than the remaining three in the tribe Delphacini. In *Purohita cervina* the dorsal and ventrolateral rows are large and of the same size. The anterior and posterior rows are small and the spines are located well apart basally. At the hind region of tibia the spines are scattered. A pair of thin, long distally tapering apodemes are articulated to the tibial base similar to that of the trochanteral base extending into the femur to which are articulated the tibial muscles. Distally, the tibia has a concavity within which is articulated the tarsus.

The tarsus (Fig. 10, Ta) is considerably shorter than the tibia and is subdivided into three tarsomeres. Of the three, the basal two are equal in length while the distal one is longer and approximately equal to the combined length of the basal two. The tarsus is clothed with large, scattered spines. The first tarsomere distally bears a crescent-shaped concavity within which fits the second tarsomere. The second one is hinged to the third by a similar articulation.

Similar to that of the tibial basal rim, the tarsus base has a pair of distally tapering apodemes for the attachment of tarsal muscles.

The pretarsus (Figs. 10, 12 and 13) arises from the end of the tarsus. To accommodate the pretarsus, the last tarsomere has undergone certain modifications. The pretarsus consists of a pair of lateral claws (Cla) and a median lobe, the arolium (Ar.) The claws are hinged to a dorsomedian process at the distal end of the last tarsomere, the unguifer (Uf). The claws are hollow, curved, distally tapering structures. Beneath the bases of claws there are small plates called auxiliae (Ax). The arolium is a small, sclerotised sac. On its ventral surface the pretarsus bears a median basal plate, the unguitractor (Ut), which is partly invaginated into the distal end of last tarsomere. To its proximal end is joined a long apodeme with which is attached the muscles of claws.

The middle legs are similar in structure to the fore legs as already mentioned. The fore coxa is longer than the middle coxa, while the fore tibia is shorter than the middle tibia.

The hind legs (Fig. 14) are remarkable for their large size in comparison to the two anterior pair of legs. The coxa (Figs. 2, 3, 9 and 14) of the hind leg is highly modified, it is short and broader than long. Posteriorly it is articulated with the trochanter by means of two small, projecting areas. This type of hinge permits a wider area of movement to the trochanter than the type of articulation found in the fore leg. The coxa is hollow and at the outer margin there is a large spine (Sh). It is firmly joined to the metapleuron and consequently the trochantin has disappeared. There is a basicostal suture, a basicosta and a basicoxite similar to that of the fore leg. In addition to this, the coxa is strengthened by a complicated system of ridges which prevent it from collapsing. In *Purohita cervina* it has small spines scattered all around, but it is without spines in the remaining two species.

The trochanter (Figs. 9 and 14, Tr) is a small ring-shaped segment. Its proximal and distal margins are highly sclerotised so much so that its internal extensions reduce the internal space. At the proximoventral region, the rim is specially thickened and from this arises a thinly sclerotised structure gradually increasing in thickness as it (Cap) projects through the hollow coxa in the metathorax. The inner end of this apodeme is circular and rimmed by three narrow sclerites (sr) which can be easily separated out in specimens boiled in potassium hydroxide solution. Muscles from the thorax are inserted to it. The trochanter is firmly united with the femur and moves along with it. The hind legs work together during jumping so as to enable the insects to take longer leaps. Like the trochanters of fore and middle legs, the hind trochanter is sparsely clothed with small spines.

The femur (Fig. 14, Fe) is shorter than the tibia and forms a distinct head proximally. The trochantero-femoral joint is so fixed that it allows only restricted movements and the trochanter moves along with the femur. The distribution of spines on the femur is similar to that of the fore leg.

Tibia (Fig. 14) is long, slender and distally broad in all the species. It has a large basal, a middle and five distal spines. The distal spines gradually decrease in size from one end to the other and occur in clusters of four and one. Posterior to these spines, the tibia bears a spur whose shape is of utmost significance in systematics. In *Liburnia pallescens* and *Delphacodes propinqua* it is foliaceous and its hind margin carries a number of teeth; fourteen to twenty in the former and sixteen to twenty five in the latter. In *Purohita cervina* the spur is cultrate and without teeth. Towards the posterior border, the spur is provided with minute

spines which are comparatively larger in *Purohita cervina*. The tibia has five rows of spines similar to that of fore tibia but smaller in size.

The first tarsomere (Fig. 14) bears at the hind margin two clusters of large spines in *Liburnia pallescens* and *Delphacodes propinqua*, one cluster with five spines gradually decreasing in size from one end to the other and the other cluster with two spines. In *Purohita cervina* it has only one cluster of six spines of unequal size. The second tarsomere bears at the hind margin one cluster of three spines of unequal size and another independent spine. There is difference in the comparative length of the tarsomeres with that of the fore leg, the basal one being longer than the other two. All over the surface, the tarsomeres bear long scattered spines. The pretarsus is similar in structure to that of the anterior pair of legs.

It is remarkable to observe that the following modifications, undergone by the hind legs, are in direct relation with the jumping mechanism :

1. The coxa of the hind leg is hollow, immovably attached to the metapleuron and strengthened by a number of ridges within it.
2. The trochanter is articulated proximally with two small conical projections of the coxa, giving maximum freedom of movement, thus compensating for the fusion of coxa with metapleuron.
3. The apodeme arising from the trochanter is enlarged chiefly to provide adequate base for the insertion of the muscles from the thorax.

#### The wings :

Wings show polymorphism in araeopids and are found to occur in three different forms, viz., brachypterous, koelopterous and macropterous. In *Delphacodes propinqua* the wings are dimorphic—macropterous as well as koelopterous; in the other two species only macropterous forms have so far been collected. Tegmina or fore wings are of slightly harder consistency, longer and narrower than the hind wings. The former are variously coloured in different species, the latter are invariably transparent.

Metcalf interpreted the wing-venation of certain families of Fulgoroidea, using the Comstock-Needham system of vein nomenclature. A decade later Muir (1923) briefly discussed the venation of Fulgoroidea, and recently Fennah (1944) has studied it in details. The wing-venation is comparatively well developed in Araeopidae. Unlike that of certain families of Fulgoroidea, such as the members of the families Flatidae, Ricaniidae etc., there is no precostal region in araeopids. In tegmen (Fig. 15), the costa (C) is a well developed, unbranched vein running along the anterior margin. Subcosta forms a common stalk basally with the costa and distally branches into two—(Sc 1) and (Sc 2). Radius coalesces with the subcosta (Sc+R) for nearly half of its length, diverges from it and after some distance bifurcates into the radial one (R 1) and the radial sector (Rs). Media (M) arises independently from the base and soon after its origin, it coalesces with the combined subcosta and radius (Sc+R) for a short distance. Distally it has three branches—(M 1), (M 2) and (M 3); according to Metcalf (1913) in Fulgoroidea the third branch (M 3) joins with another (M 4) during the course of the development of media. Cubitus is forked into two, namely, (Cu 1) and (Cu 2), soon after its origin; the former branch distally bifurcates to give the (Cu 1a) and (Cu 1b). The anal vein is two branched, (IA) and (IIA); they distally fuse to give a 'Y' vein, a characteristic feature of the superfamily Fulgoroidea (Imms, 1957). The first anal (IA) represents the postcubitus of Snodgrass (1935) because of its

independent origin without fusing either with the base of the cubitus or with the third axillary. As the revised nomenclature of Snodgrass (1935) in respect of the cubito-anal region, has not met with general acceptance the authors have preferred to retain the old name, the cubitus two (Cu 2). All the veins of tegmen, except cubitus two are characterised by the presence of macrotrichiae throughout their length. In *Purohita cervina* most of the macrotrichiae are found on either side apposing the veins and not always on the veins.

The hind wing (Fig. 16) is conspicuous for the absence of macrotrichiae and the enlarged anal area. The costa is similarly disposed as the tegmen. The subcosta is unbranched and runs very close to the costa. The radius is represented by a single vein. The media is reduced and is also represented by a single vein. Basally the subcosta, the radius and the media all unite together and run for a short distance apposing the costa anteriorly. Proximal to this the media diverges to join with its basal sclerite, the combined subcosta and radius unite with its own sclerite; the costa continues independently. The branching of cubital vein is similar to that of the tegmen but in the hind wing the (Cu la) coalesces with the media for almost its entire length being separated only near the distal margin. The first anal is unbranched, the second anal is three branched (IIA 1, IIA 2 and IIA 3) to support the enlarged anal area.

The articular membrane of the wings is thickened and corrugated at its posterior margin to form the axillary cord. The pteralia of the tegmen (Fig. 15) are the following :

**Tegula (Tg).** It is a well developed sclerotised plate overlapping the base of the tegmen and movably articulated with the base of the costal vein. It is provided with scattered, small hair on the surface.

**Humeral plate (Hp).** It is a small, sclerotised conical plate projecting anteriorly and situated at the base of the costal vein. It is rooted over by the enlarged tegula.

**First and second axillaries (Fa).** The first and second axillaries are fused together to form a small sclerite situated between the costa and the median plate. It is slightly curved and is in connection with the fused subcostal and radial veins distally.

**Median plates (Mps).** They consist of a pair of unequal, apposing small sclerites, the larger sclerite lying proximal to the smaller. These are situated between the fused first and second axillary and the third axillary. The media is in connection with the distal sclerite.

**Third axillary (III Ax).** It is a small triangular sclerite situated at the posterior margin of the tegmen in between the median plates and the axillary cord. Medially it touches the proximal median plate.

The relative position of the sclerites in the articular membrane of the hind wing (Fig. 16) is similar to that of the fore wing, though they differ in shape and size. The humeral plate and the tegula are reduced. The median plate (Mp) is represented by a single large plate. The third axillary (III Ax) has proportionately increased in size along with the increased importance assumed by the second anal vein in the hind wing.

#### **Discussion :**

Aracopids exhibit great morphological diversity in the thorax and particularly marked in the metathorax. Their pro and mesothorax very much resemble



those of other fulgoroids except in the presence of a number of secondary sutures in the latter. The development of the secondary sutures in the thorax of these insects, which lack corresponding ridges, is interesting as it follows a similar course in both the tribes under observation, namely, Delphacini and Tropidocephalini, suggesting similar lines of evolution.

In *Liburnia pallescens* and *Delphacodes propinqua* the first and third phragmata show sexual dimorphism. In males, the first phragma is comparatively well developed and the third phragma much larger than the corresponding phragma of females. So the males are adapted for better flying in the subfamily Delphacini. In *Purohita cervina* the phragmata are uniformly developed in both the sexes.

In araeopids, the jumping mechanism is developed in connection with the hind legs, although the metathorax lacks furca. In order to compensate this loss, the metathorax has developed a complicated system of ridges to form an internal skeletal frame work at the lateral sides, to withstand the extra stresses and strains shouldered by it.

In araeopids the apodeme (Fig. 9, Cap) arising from the ventral side of the trochanter and projecting into the metathorax in connection with the jumping mechanism, has extraordinary importance. Qadri and Aziz (1950) report an analogous case in *Pyrilla perpusilla* in which they attribute its origin to the coxa. In araeopids this apodeme is homologous to the apodeme arising from the trochanteral base for the muscular attachment of the trochanter in the fore and middle legs. The development of the jumping mechanism has its own impact on the hind leg as a whole as it has grown enormously in all directions than the fore and middle legs. The hind coxa is also, consequently, immovably articulated to the pleuron, and has developed two distal conical projections for the attachment of the trochanter thus allowing maximum freedom of movement to the latter so much so that it can swing on the former.

The function of the spur has been a matter of speculation in araeopids. Some early workers believed that it assists in some way or other to take longer leaps, but other homopterans without spur also leap equally well, if not better, and so the spur does not appear to have any direct bearing with the jumping mechanism. The authors suspected that these insects must be making use of their spurs for some such purpose as cleaning because of their structure and position, but their observation in the field and experiments in captivity have failed on *Liburnia pallescens* and *Delphacodes propinqua* to exhibit any such action on the part of the insects under study.

### Summary :

The present paper deals with the morphology of the thorax of three species of Araeopidae belonging to two tribes viz., tribe Delphacini—*Liburnia pallescens* and *Delphacodes propinqua* and tribe Tropidocephalini—*Purohita cervina*. The following features are noteworthy.

(1) In araeopids the pro and mesothorax are similar to other fulgoroids except for a number of secondary sutures for the latter. The metathorax exhibits great morphological diversity from other allied forms and develops a number of secondary sutures. It lacks furca and has a complicated skeletal frame work at the lateral sides. In the tribe Delphacini, the first and second phragmata exhibit sexual dimorphism, whereas in Tropidocephalini they are uniformly developed in both the sexes.

(2) The hind leg is much larger than the fore and middle legs and bears the spur, the characteristic feature of the family. The spur is foliaceous with a num-

ber of teeth at the posterior margin in *Liburnia pallescens* and *Delphacodes propinqua*, whereas it is cultrate and without teeth in *Purohita cervina*. The hind legs develop a jumping mechanism.

(3) In tegmen, the costa in single, subcosta two branched, radius is represented by radial one and radial sector, media three branched, cubitus two branched, of which the cubitus one in its turn is bifurcated distally, and the anal vein is two branched which join distally to form the characteristic 'Y' vein of Fulgoroidea. In the hind wing, costa is similar to the fore wing, subcosta, radius and media unbranched, cubitus and first anal similar to the fore wing, and the second anal three branched. The first and second axillaries are fused together in both the wings, the median plates are two in the tegmen and one in the hind wing, the third axillary is much larger in the hind wing.

#### Acknowledgments :

The authors express their grateful thanks to Dr. R. K. Singh, M.A., LL.B., D.Ed. (Harvard), Principal, Balwant Rajput College, Agra, for providing research facilities.

#### Literature Cited :

- Akbar, S. S. The morphology and life history of *Leptocoris varicornis* Fabr. (Coreidae, Hemiptera)—A pest of paddy crop in India, Part I, Head and Thorax. *Ali. Musl. Uni. Publ. (Zool. Ser.), Ind. Ins. Typ.* pp. 53, 1957.
- Crampton, G. C. A contribution to the comparative morphology of thoracic sclerites of insects. *Proc. Acad. Nat. Sci. Philadelphia*, 3-54, 1909.
- Fennah, R. G. The morphology of the tegmina and wings in Fulgoroidea. *Proc. Ent. Soc. Washington*, 46 (7) : 185-198, 1944.
- Imms, A. D. A General Text Book of Entomology. Methuen and Company Ltd., London, pp. 762, 1957.
- Martin, J. F. Thorax and cervical sclerites of insects. *Ann. Ent. Soc. America*, 9 : 35-83, 1916.
- Mathur, P. N. and A. N. T. Joseph. Studies on the external morphology of *Peregrinus maidis* (Ashmead) (Homoptera, Fulgoroidea, Araecopidae=Delphacidae), Part II, Thorax. *J. Anim. Morph. Physiol.*, 8 (2) : 81-92, 1961.
- Metcalf, Z. P. The wing venation of the Fulgoroidae. *Ann. Ent. Soc. America*, 6 (3) : 341-352, 1913.
- Muir, F. On the classification of Fulgoroidae. *Proc. Haw. Ent. Soc.*, 5 (2) : 215-218, 1923.
- Myers, J. G. Morphology of Cicadidae. *Proc. Zool. Soc. London*, 25 : 365-472, 1928.
- Qadri, M. A. H. and Abdul Aziz. Biology, life history, and external and internal anatomy of *Pyrilla perpusilla* Walker. *Ali. Musl. Uni. Publ. (Zool. Ser.), Ind. Ins. Typ.*, pp. 33, 1950.
- Snodgrass, R. E. The thorax of insects and the articulation of the wings. *Proc. U. S. Nat. Mus.*, 36 : 511-593, 1909.
- Snodgrass, R. E. Principles of insect morphology. McGraw-Hill Book Co., N. Y., pp. 667, 1935.

# EFFECT OF CAFFEIN ON FLOWERING OF CALENDULA OFFICINALIS—A NOTE

By

D. M. VERMA

*Botanical Survey of India, Allahabad*

[Received on December 1, 1962]

The paper deals with certain observations based on exploratory experiments performed at the Central Botanical Laboratory, Allahabad, to evaluate the effect of caffein on the flowering behaviour of *Calendula officinalis* L.—an important ornamental plant.

128 seedlings of *C. officinalis* were transplanted on 20-12-60 to two comparable field plots. In one of the plots, seedlings were sprayed weekly with 100 ppm. aq. sol. of caffein and those of the other plots were sprayed with distilled water. The treatments started on 27-12-60, while the plants were in 4-6 leaf stage. In all three treatments were given. Record of the time and number of flowers produced was maintained throughout the flowering period. Last observation was made on 11-4-61 by which time both the sets of plants had completed their life cycle.

Table 1 gives the data on floral initiation, total number of flowers produced in treated and control plants.

TABLE 1

Spray treat- ments	Total number of plants	Date of flower initiation	Flowering period	Total number of flowers	No. of flowers/ plant
Caffein 100 ppm.	64	20-1-61	20-1-61 to 11-4-61	1965	30·7
Control (distilled water)	64	24-1-61	24-1-61 to 11-4-61	1359	21·2

The results indicate a slightly earlier swing from vegetative to reproductive phase and a remarkable increase in the flowering capacity of treated plants. Daily records show a gradual increase in the average number of flowers produced per plant. Observations did not reveal any change in the vegetative growth or age of the plants.

# ROLE OF MANGANESE IN THE GROWTH OF ROOT AND SHOOT OF *CAJANUS CAJAN*

By

AMAR SINGH and ARCHANA PAL

*Department of Botany, University of Allahabad*

[Received on December 1, 1962]

## Introduction :

Manganese played a significant role in the nutrition and physiology of plants through its influence on glycolysis (Lardy, 1949); nitrogen metabolism (Smith 1951, Hewitt, 1951; and Delwische *et al* 1951); organic acid metabolism (Ochoa, 1948; Bonner, 1950); nitrate reduction (Mendel and Visser, 1951); formation of ascorbic acid (Rudra, 1939; Hivon *et al* 1951) and photosynthesis (Hiltner, 1924; Gerretsen, 1949).

Manganese was also intimately associated with the activity of certain enzymatic reactions. Its stimulating effect on plant growth was recorded by Loew (1903), Bertrand (1905), Máze (1914) and others. The many-sided action of manganese was inter-related to the supply of copper, phosphorus, nitrogen and iron to plants (Mulder and Gerretsen, 1952) and was also modified by light intensity (Hanner, 1945).

In spite of such wide functions and associations of manganese with growth of the plants the question of its indispensability has been doubted. Glasstone (1947), working with tomato plant, reported that manganese was not required for root growth. A few years later, Burstrom (1950) suggested the essentiality of manganese for the normal growth of roots, its specific influence on root in contradistinction to that on shoot remained obscure.

## Methods and Materials :

These investigations on the comparative effect of varying levels of manganese supply on *Cajanus cajan* with constant supply of iron were taken up with a view to elucidate its action on the light-loving (shoot) and light-avoiding (root) parts of the plant. Three seedlings were raised in each polythene container containing acid-washed silica sand and nutrients as detailed in an earlier paper (Singh and Pal, 1963).

Manganese was supplied in three doses, Mn(n), Mna and Mnb corresponding to 0.01, 1.25 and 2.50 ppm of Mn respectively. Alongside, a manganese deficient (-Mn) series was also maintained.

The treatments referred to herein denote the Fe : Mn ratio as below :

-Mn  $\equiv$  no manganese supply, only Fe present to the extent of 0.077 ppm.

Mn(n)  $\equiv$  normal Mn supply; 0.077 ppm of iron and 0.01 ppm of manganese, Mn : Fe :: 1 : 7 (approx.).

Mna  $\equiv$  the ratio of Mn : Fe :: 16 : 1 (approx.) with 0.077 ppm of Fe and 1.25 ppm of Mn.

Mnb  $\equiv$  the ratio of Mn : Fe :: 32 : 1 (approx.) with 0.077 ppm of Fe and 2.50 ppm of Mn.

The supply position of manganese varied while that of iron remained static. Manganese was supplied in the form of manganese chloride and iron as iron sulphate.

Morphological variations of growth of shoot and root *viz*, branching or ramification, linear growth and also dry matter accumulation, tissue moisture, sugar accumulation as well as amino acid content were assessed.

The two dimensional chromatographic technique of Consden, Gordon and Martin (1944) was followed using 28×28 cm Whatman No. 1 chromatography filter paper. The extract was spotted 3 cm away from each of the sides of the chromatography paper. Partridge's solvent, as modified by Fowden (1954), containing phenol saturated with 0.5% (V/V)  $\text{NH}_4\text{OH}$  solution was used as first solvent. Dry chromatograms were then developed in the second solvent *i.e.* n-butanol, acetic acid and water (4 : 1 : 5) by the other side, and allowed to dry. Ninhydrin 0.1% (W/V) in normal butanol was sprayed to locate the spots. The dry chromatograms were heated at 85°C for 30 minutes in an oven and later exposed to room conditions for 30 minutes.

The average figures from replicates have been presented.

### Experimental Findings.

#### Branching Behaviour :

*Shoots.*—Branching of shoots was not observed in any of the treatments at any stage, upto the 41 day age of the plants (Plate 1).

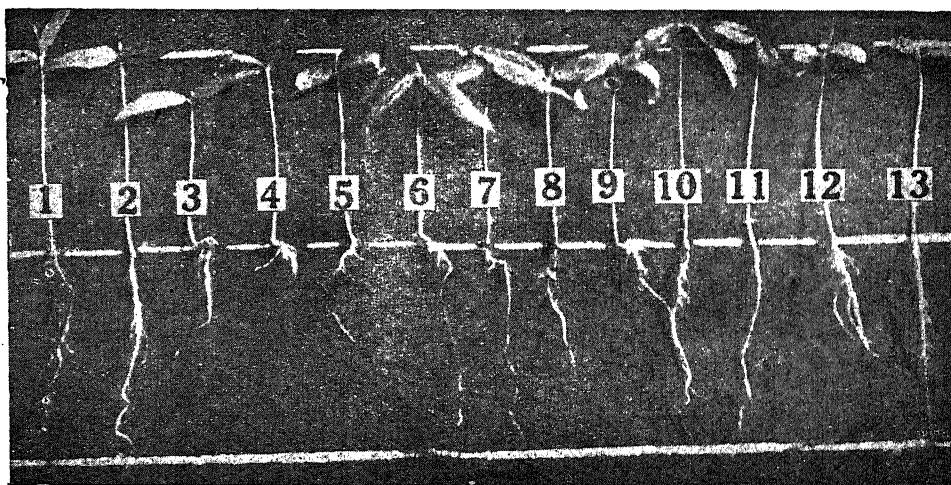


PLATE 1. Effect of varying levels of micronutrient supply on the growth of *Cajanus cajan* at 34-day age. 1 : Ba, 2 : Bb, 3 : —B, 4 : Zna, 5 : Znb, 6 : —Zn, 7 : Mna, 8 : Mnb, 9 : —Mn, 10 : Moa, 11 : Mob, 12 : —Mo, 13 : control Mn(n).

*Root.*—Branching in roots, was stimulated by manganese feeding Mnb dose produced maximum number of roots at all the stages of observation though it fell after 27 days (Table 1).

TABLE 1  
Effect of levels of manganese supply on ramification of roots  
(No./Plant)

Age (days)	Levels of supply			
	-Mn	Mn(n)	Mna	Mnb
20	20.00	17.00	9.60	25.30
27	20.33	18.30	25.00	31.00
34	19.66	22.25	17.66	25.50
41	19.00	24.30	..	25.00

The manganese deficient series was not of much significance; root number showed irregular response in its behaviour such that manganese shortage was unfavourable at the 41-day age. Incidentally it may be of interest to note that no-Mn series appeared to be better than its normal supply as well as Mna series on the first day of observation. The normal supply induced progressive increase in the ramification of roots and closely followed the Mnb series on the last day of observation (Table 1).

#### Linear growth :

*Shoots.*—The effect of manganese concentrations on linear growth of shoot varied. Plants fed with normal amount of manganese brought about maximum increase in shoot length throughout the period of observation except at the 27-day age when manganese starved plants excelled (Table 2).

TABLE 2  
Effect of levels of manganese supply on the linear growth of shoot  
(cm/plant)

Age (days)	Levels of supply			
	-Mn	Mn(n)	Mna	Mnb
20	6.30	8.00	6.50	7.61
27	10.30	11.66	10.05	9.70
34	11.60	12.57	12.13	10.40
41	11.83	13.02	..	10.65

The supply of manganese to the tune of 2.5 ppm (Mnb) proved deleterious to shoot length after the 20-day age of the plants.

The leaves of the manganese-free series showed deficiency symptoms in the form of chlorosis starting from the edges finally spreading all over in a haphazard pattern from the 27-day age onwards. It might be of interest to note that under similar levels of boron supply *Cajanus cajan* did not exhibit symptoms of toxicity. It remained to be ascertained, however, if the plant could tolerate higher quantities of boron or its selective action on the light-loving and light-avoiding parts of the plant, as stressed by Singh and Pal (1963), was responsible for the behaviour, a role that manganese could not display.

*Roots.*—Similar to shoot, root length also showed maximum length under conditions of normal Mn supply throughout but for the 34-day age when it was exceeded by manganese-free and the Mna feeding. On the last day of observation

the response of Mn(n) was closely followed by the minus Mn series in this respect due to the increased rate of elongation during the 20-41, day period in the latter. The Mn-deficient plants possessed, markedly shorter roots than the control ones at the initial stage (Table 3).

The effect of Mn(a) treatment could not be recorded further due to the destruction of the plants of the series by rats on the 35th day. Mnb series possessed somewhat shorter roots than others.

TABLE 3  
Effect of levels of manganese supply on the linear growth of roots  
(cm/plant)

Age (days)	Levels of supply			
	-Mn	Mn(n)	Mna	Mnb
20	5.20	9.30	6.70	5.50
27	12.20	12.30	9.75	8.60
34	13.00	12.70	13.00	12.00
41	13.80	14.62	. .	12.00

#### Dry matter accumulation :

*Shoots.*—Progressive increase in dry matter production of shoots was registered in the series receiving higher doses of manganese, the rise being most marked in the 27-34 day period in Mnb series (Table 4). On the last day of observation, maximum amount of dry matter had accumulated in the maximum supply of Mn. The plants receiving deficient as well as normal supply of Mn showed a fall in dry matter augmentation during the 20-27 day period. Manganese deficiency brought about greater augmentation of shoot dry matter than its maximum dose at the 20-day age. It also depicted that at the initial stage Mnb level of supply proved detrimental to dry matter production. After the 27-day age there was a fall in dry matter accumulation indicating that the need for manganese was greater after that stage. It was further supported by the fact that during the period (27-34 days) the maximum dose of Mn supply augmented higher rate of dry matter accumulation.

TABLE 4  
Effect of levels of manganese supply on dry-matter accumulation in shoot  
(gm/plant)

Age (days)	Levels of supply			
	-Mn	Mn(n)	Mna	Mnb
20	0.0400	0.0490	0.0320	0.0360
27	0.0400	0.0380	0.0350	0.0367
34	0.0378	0.0400	0.0400	0.0492
41	0.0372	0.0430	. .	0.0500

Both the control and the Mna level of supply showed an increase in dry matter during the 27-34 day period, the latter excelled the former in effectiveness.

*Roots.*—The no-manganese treatment augmented low dry matter accumulation at the 20-day age, it being thereafter marked by a rapid increment such that on the

27th day it reached the peak of dry matter production to be followed by a decline giving a walkover the Mna and Mnb treatments (Table 5). Between the initial stage of 20-27 days the Mn(n), Mna and Mnb treatments seemed to have minimum variation in effect.

TABLE 5  
Effect of levels of manganese supply on dry matter accumulation in root  
(gm/plant)

Age (days)	Levels of supply			
	-Mn	Mn(n)	Mna	Mnb
20	0.0040	0.0055	0.0050	0.0050
27	0.0094	0.0066	0.0066	0.0062
34	0.0076	0.0075	0.0083	0.0065
41	0.0080	0.0076	. .	0.0082

Roots depicted a higher hydration ratio than the shoots in the Mn fed plants at all the stages of observation (Fig. 1, 2) in a manner similar to that seen under varying supplies of boron (Singh and Pal, 1963). The normal supply of Mn (Mn : Fe :: 1 : 7) showed gradual increase in the hydration ratio of the roots whereas Mnb and -Mn treatments showed decrease with advance in age. In the shoots, however, increase in the hydration ratio was evidenced under all the treatments. Considering the plant as a whole hydration ratio increased with age in the treatments, the minimum variation being shown in the widest Mn : Fe ratio. The hydration ratio of the roots was higher than of roots, more so, when considered on the basis of the dry matter production of the plants.

#### Accumulation of sugars :

*Shoots.*—Sugar percentage increased with age except in the control and the manganese starved plants; the former showed a decrease in both reducing and non-reducing sugars during the 34-41 day period and the latter in non-reducing sugar only, during the 27-34 day period (Table 6).

TABLE 6  
Effect of levels of manganese supply on the accumulation of sugars in shoot  
(mgm/gm)

Reducing				Sugar	Non-reducing			
Levels of supply					Levels of supply			
-Mn	Mn(n)	Mna	Mnb	Age (days)	-Mn	Mn(n)	Mna	Mnb
98.55	251.10	29.60	453.60	27	112.05	85.05	37.80	10.80
205.87	405.00	69.00	465.75	34	87.75	199.00	60.75	67.50
211.50	239.50	. .	753.30	41	108.00	140.40	. .	151.20

The quantity of reducing sugar was maximum in the Mnb treated series throughout the period of observation and significantly so with increase in age. But for the 27-day age non-reducing sugar also was maximum in the case of Mnb level of supply.



**Roots.**—Plants with normal supply of manganese had maximum amount of reducing sugars in the roots at all the stages of observation (Table 7). Supra supply of manganese and also the manganese deficient series could accumulate equal amounts of reducing sugars at the 41-day age though at the initial stage manganese deficient series possessed much less quantity of reducing sugars as compared to other treatments.

Non-reducing sugar was in maximum quantity in the Mnb treated series as in the case of shoots, throughout the period of observation. Manganese deficiency as well as its normal supply were noted to possess same amount of non-reducing sugar at the initial and at the last day of observation. (Table 7).

TABLE 7

Effect of levels of manganese supply on the accumulation of sugars in root (mgm/gm)

Reducing				Sugar	Non-reducing			
Levels of supply					Levels of supply			
-Mn	Mn(n)	Mna	Mnb	Age (days)	-Mn	Mn(n)	Mna	Mnb
0.675	2.700	1.350	1.350	27	0.675	0.675	1.012	1.012
1.350	2.700	1.687	2.025	34	0.675	0.337	1.012	1.687
2.360	2.712	. .	2.362	41	1.012	1.012	. .	2.025

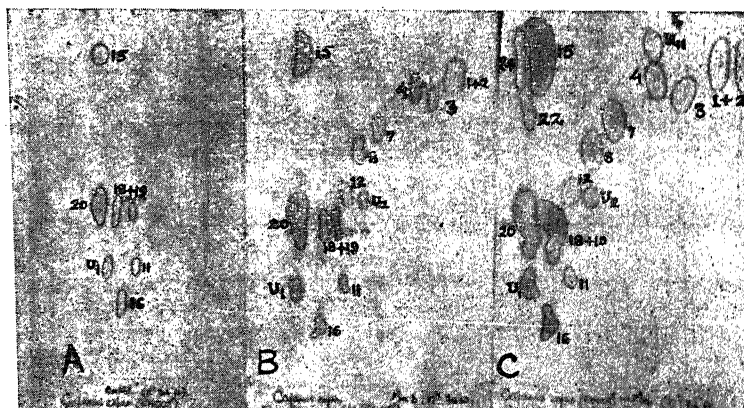


Plate 2. Photographs of two dimensional chromatograms showing the position of various free amino acids present in shoots of *Cajanus cajan* at the age of 27-days under the influence of manganese feeding. A-Mn(n) ; B-Mnb ; C-Mn.

#### Amino-acids :

**Shoot.**—Maximum number of free amino acids, viz., Leucines and phenylalanines, valine,  $\gamma$ -amino butyric acid  $\alpha$ -alanine,  $\beta$ -alanine, glutamic acid, threonine, arginine, aspartic acid, glycine-serine, lysine as well as histidine were recorded

at the 27-day age in the -Mn series of plants (Table 8). In contrast to this, in Mn(n) supply fewer free amino acids namely glutamic acid, arginine, aspartic acid and glycine-serine were detected and these were present in smaller intensity.

TABLE 8  
Effect of levels of manganese supply on the amino acid content of shoot of *Cajanus cajan*

Treat- ments	Age (days)	Amino acids present*																	
		1+2	3	4	5	7	8	11	12	15	16	18, 19	20,	21	24	U <sub>1</sub>	U <sub>2</sub>	U <sub>8</sub>	U <sub>11</sub>
-Mn	27	++	++	++	+	++	++	+++	++	++++	+++	++++	-	++	-	+++	++	-	+
	34	-	-	-	-	+	++	++	-	+	+++	+++	+++	+++	-	-	-	-	-
	41	++	++	+++	-	++	+++	-	-	+++	++	+++	++++	-	+	-	-	++	-
Mn(n)	27	-	-	-	-	-	-	+	-	+	+	++	-	-	-	-	-	-	-
	34	++	-	-	-	++	++	+	-	+++	++	++	+++	-	-	-	-	-	-
	41	++	-	-	-	+++	++	++	-	+++	+++	++	+++	-	-	-	-	-	-
Mna	27	+	+	+	-	+	++	+	-	+	+	-	-	-	-	+	+	-	-
	34	-	-	-	-	-	+	+	-	-	++	++	++	++	-	-	-	-	-
	41	SAMPLES NOT AVAILABLE																	
Mnb	27	+	+	+	-	+	+	++	+	++	++	++	-	-	-	++	-	-	-
	34	-	-	-	-	-	-	+	-	+	+	++	+	-	-	-	-	-	-
	41	++	+++	-	-	++	+++	-	-	++	++	+++	+++	-	+	-	-	-	-

\*1+2. Leucines and phenylalanines; 3. valine; 4.  $\gamma$ -amino butyric acid; 5. tyrosine; 7.  $\alpha$ -alanine; 8.  $\beta$ -alanine; 11. glutamic acid; 12. threonine; 15. arginine; 16. aspartic acid; 18-19. glycine-serine; 20. asparagine; 22. histidine; 24. lysine; *Rf* values of unidentified free amino acids: U<sub>1</sub> 0.270; U<sub>2</sub> 0.492; U<sub>11</sub> 0.900.

The Mna dose of supply showed the presence of  $\alpha$ -alanine,  $\beta$ -alanine, glutamic acid, arginine and aspartic acid whereas Mnb of leucines, valine,  $\gamma$ -amino butyric acid,  $\alpha$ -alanine,  $\beta$ -alanine, glutamic acid, threonine, asparagine, aspartic acid glycine-serine at the 27-day age.

Analysing the effect of the change in Mn supply at this stage of observation, lysine and histidine were found to be present only in the manganese deficiency series whereas threonine in both the -Mn and Mnb series.

The absence of glycine-serine was marked in the Mna level of supply. The concentration of the amino acids present was always higher in the plants fed on manganese deficient nutrient (Plate 2).

The free amino acid content of the shoot declined definitely at the next stage as against the first one in the -Mn, Mna and Mnb concentration though not in the control, where more free amino acids were present. Later the number of free amino acids increased in all the treatments, except in the control,

## Discussion :

Plants fed on Mn : Fe :: 1 : 7 ratio (Mnn) depicted a normal healthy appearance and good performance. Lal and Rao (1953) reported improvement in yield and all external growth attributes with Mn : Fe ratio as 2.0-2.5 in the case of sugarcane. It has long been ascertained by Bertrand (1897) that the ratio of Mn to Fe was more important, than the absolute concentration of manganese, in the proper functioning of plants. Plants raised in deficiency (but for the quantity present in seeds) manifested lesser development of roots as against the Mn(n) treatment.

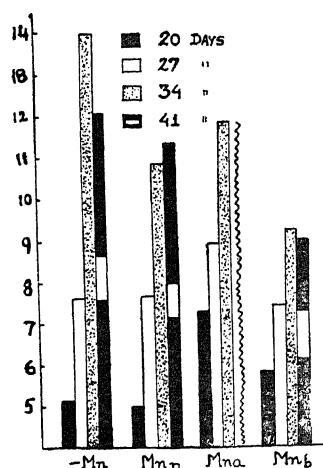


Fig. 1

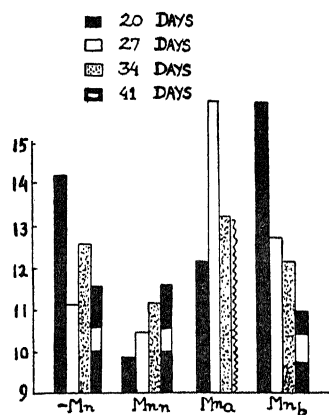


Fig. 2

Fig. 1. The effect of varying levels of manganese supply on the hydration ratio of shoots of *Cajanus cajan* at different periods of life cycle.

Fig. 2. The effect of varying levels of manganese supply on the hydration ratio of roots of *Cajanus cajan* at different periods of life cycle.

Poor root development, in the deficiency of Mn, had been reported by Samuel and Piper (1929) specially in alkaline soils. The reaction of the medium of growth being non-alkaline the ill-effects were not marked. Again, no microbiological disintegration as reported by Lal and Rao (1953) was noted possibly due to the fact that small amounts of manganese available in the seed and additional supply of 0.01 ppm of manganese delayed the effects as pointed out earlier by Lundegardh (1932) and Gerretsen (1937). Another reason for the non-appearance of the symptoms in the roots was, as pointed out by Haas (1932), that root symptoms developed later than those in leaf. In these investigations deficiency symptoms were noted in the leaves only after the 27-day age; with advance in age greater need for manganese was felt since under its shortage there was a notable decrease in root length as well as its number. Along with the absolute number the rate of increase in length also declined (Tables 1 and 3). The no-Mn series was predominantly marked in the fall in the dry matter accumulation in the roots (Table 5).

Manganese was reported to affect dry matter production in the case of paddy and barley by Lal and Rao (1953). Plant growth and dry weight has been noted to be well related to manganese supply position in these investigations.

Presumably, hydration ratio was responsible for higher dry matter accumulation in both the light-loving and light-avoiding parts of the plant, (Figs. 1, 2) confirming the findings of Kelly (1947) that the hydration ratio was an index of the availability of carbohydrates. The relationship between the hydration ratio of the entire plant to the dry matter produced was shown to be affected by change in Mn : Fe supply position. In the plants raised on Mn : Fe ratio of 1 : 7 the hydration ratio/D.W. increased with age whereas with widest Mn : Fe ratio of 32 : 1 the hydration ratio/D.W. values decreased progressively. Thus the role of Mn : Fe ratio of feeding influenced the water utilisation by plant *vis-a-vis* its dry matter production.

The Mn : Fe ratio as 32 : 1 proved optimum for the ramification of roots, shoot dry weight, reducing and non-reducing sugar of both root and shoot. The Mn/Fe balance played an important role in the growth of plants as also reported by Shive (1941) and Sommers and Shive (1942).

Active iron to active manganese ratio of the total concentration of the elements seemed to be favourable to the accumulation of sugar. Reduced content of sugars and starch in leaves of oat (McHargue, 1926) and of tomatoes (Eltinge, 1941) deficient in manganese provided a direct evidence of reduced photosynthesis was well supported by these findings.

Change in the level of manganese supply induced variations in free amino acid content of the shoot both in extent and kind. Larger number of free amino acids in conditions of manganese deficiency pointed out to the relative accumulation of amino acids under deficiency conditions as also suggested by Steinberg (1953).

Sommers and Shive (1942) laid greater significance on the ratio of iron to manganese rather than absolute concentrations though Nicholas (1949) reported that iron manganese ratio was not of much significance in determining the status of field crops. In view of the results obtained with varying iron to manganese ratios, it might well be argued that not all the elements supplied got into the plant body and that only a reasonable amount entered their metabolic cycle. This might be conditioned by the supply position of phosphates, nitrogen, copper, iron etc. governing the effect of manganese as reported out by Mulder and Gerretsen (1952).

The ratio of manganese : iron when reduced to half i.e., 16 : 1 proved more conducive to root elongation, its dry matter accumulation, shoot elongation and also the fresh weight of the entire plant and its light-loving and light-avoiding components also. The data suggested that the photosynthetic activity of the *Cajanus* plants was at its top under the widest Mn : Fe ratio tried. The Mn:b fed plants contained maximum amount of sugar in shoots again pointing to the high photosynthetic activity but its low content in roots signified that the ratio did not prove better for translocation but for accumulation in the shoots. This was due to the difference in the mode of action of the iron to manganese ratio occasioned by increase in manganese supply in the light-avoiding and light-loving parts of the plant.

Burstrom (1950) pointed out that no record of direct effect of manganese on root development nor of any specific signs of manganese deficiency in roots occurred. The present findings successfully showed that manganese affected the growth of *Cajanus cajan*, specially in the case of number of roots, shoot dry weight, elongation etc.

The combined length of plant declined in the manganese deficient series after the 34-day age. In the normal supply of manganese with 1 : 7 ratio, maximum root length was observed at the 41-day age though in the case of Mn:b, with

widest Mn : Fe ratio of 32 : 1, even greater root length was recorded at the 27-day age though not towards the end. It suggested that possibly the ratio was so wide as to prove deleterious after the 27th day though less in comparison to the manganese deficient series. It is also evidenced by the development of toxicity symptoms in respect of chlorosis of the shoots and decreased root length that the Mnb ratio did not prove very fruitful. In the matter of total dry matter, however, Mnb recorded maximum at dates subsequent to the beginning indicating increased photosynthetic activity with increase in manganese supply. The tissue moisture of the entire plant was maximum in the Mna series suggesting that the Mn : Fe ratio could tell on the water economy of plants. The water requirement of plants was reduced by increased supplies of manganese above the normal. Shoot/root ratio on weight basis was higher in Mnb treatment though the same did not hold good for shoot/root ratio on length basis (Figs. 3 and 4).

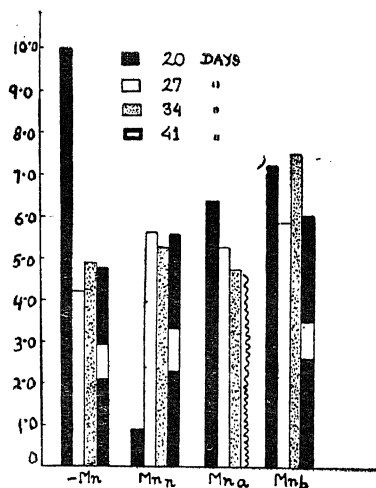


Fig. 3

Fig. 3. The effect of varying levels of manganese supply on dry matter accumulation ratio (shoot/root) of *Cajanus cajan* at different periods of life cycle.

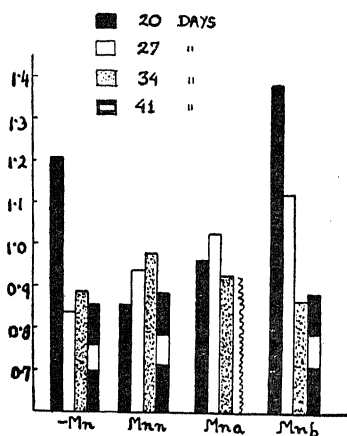


Fig. 4

Fig. 4. The effect of varying levels of manganese supply on Linear growth ratio (shoot/root) of *Cajanus cajan* at different periods of life cycle.

Elongation was associated with rapid synthesis of RNA, DNA and protein whereas dry matter accumulation was the net result of the metabolic activities. Although direct relationship between the rate of photosynthesis and root elongation was reported by Jaccard (1928) it has not been possible to confirm his findings under the conditions of these investigations.

Manganese deficiency symptoms were not aggravated possibly due to the raising of the plants in shade since *Cajanus* could flourish under shade conditions also. It also implied that the plant did not reduce its photosynthetic activity to the extent as to be detrimental to its growth at least for the initial period of 41 days. Thus in the matter of root elongation boron (Singh and Pal, 1963) and manganese played an important role and the conclusions of Burstrom (1961) that auxins could not be taken as the limiting factor in root elongation seemed to be tenable. Increase in level of manganese supply to Mnb level produced stunted roots, a behaviour opposed to the one observed with increase in the level of boron to an equal extent. It depicted that whereas the former was negatively related to

the elongation of light-avoiding parts of the *Cajanus* plant the latter was positively correlated.

### Summary :

Morphogenetic effects of increasing dosage of manganese on the light-avoiding and light-loving parts of *Cajanus cajan* (Type 1) plants under constant supply of iron at 0.07 ppm in each case have been recorded. Manganese deficiency series has also been included.

Increased supply of manganese stimulated ramification of roots. In linear growth, manganese supply of 2.5 ppm (Mn : Fe :: 32 : 1) proved deleterious for shoot as well as root; the normal supply (0.01 ppm, Mn : Fe :: 1 : 7) proved optimum. Dry matter production of both shoots and roots increased with increase in Mn supply. Widest Mn/Fe ratio of 32 : 1 proved optimum for the reducing and non-reducing sugars of the shoots, and only non-reducing sugar of the root; for reducing sugar content of the root the ratio 1 : 7 proved optimum. The accumulation of larger number of free amino acids in the shoot of the manganese deficient plants was evidenced.

### Literature Cited

- Bertrand, G. *C. R. Acad. Sci., Paris*, **124** : 1032-1035, 1355-1358, 1897.  
——— *Compt. rend.*, **141** : 1254-1255, 1905.  
Bonner, J. *Plant Biochem.*, Acad. Press, N. Y., 1950.  
Burstrom, H. Trace elements in plant physiology. Symp. organised by the International Union of Biological Science with the support of UNESCO in 1947, 1950.  
——— *Rec. Adv. in Bot.* Vol. II, Univ. Toronto Press, 1961.  
Consden, R., Gordon, A. H. and Martin, A. J. P. *Biochem. Jour.*, **38** : 224-232, 1944.  
Delwische, C. C., Loomis, W. D. and Stump, P. K. *Arch. Biochem. Biophys.* **33** : 333-338, 1951.  
Eltinge, E. T. *Plant Physiol.*, **16** : 185-189, 1941.  
Fowden, L. The nitrogen metabolism of ground nut plants. The role of  $\gamma$ -methylene glutamine and  $\gamma$ -methylene glutamic acid. *Ann. Bot.*, **18** : 417-440, 1954.  
Gerretsen, F. C. *Ann. Bot.*, **1** : 207-30, 1937.  
——— *Plant and Soil*, **1** : 346-358, 1949.  
Glasstone. *Am. J. Bot.*, p. 81, 1947.  
Hamner, K. C. *Soil Sci.*, **60** : 165-171, 1945.  
Haas, A. R. C. *Hilgardia*, **7** : 181-206, 1932.  
Hewitt, E. J. *Ann. Rev. Plant Physiol.*, **2** : 25-32, 1951.  
Hiltner, E. *Land. W. Yohrb.*, **60** : 639-769, 1924.  
Hivon, K. J., Doty, D. M. and Quackenbush, F. W. *Soil Sci.*, **71** : 353-359, 1951.  
Jaccard, P. *Jahrb. Wiss. Bot.*, **69** : 549, 1928.  
Kelly, S. *Amer. Jour. Bot.*, **34** : 521, 1947.  
Lal, K. N. and Rao, M. S. S. Micro element nutrition of plants. B. H. U. Press, 1953.

- Lardy, H. A. *Resp. Enzymes*, Burgers Publishing Co. Minneapolis, 1949.
- Loew, O. *Land. W. Jahrb.*, **32** : 437-448, 1903.
- Lundegardh, H. Die Nahzstoffnahme der Pflanze, Jena, 1932.
- Máze, P. *Inst. Pasteur Ann.*, **28** : 21-46, 1914.
- McHargue, J. S. *J. Ind. Eng. Chem.*, **18** : 172-175, 1926.
- Mendel, J. L. and Visser, E. D. W. *Arch. Biochem. Biophys.*, **32** : 158-169, 1951.
- Mulder, E. G. and Gerretsen, F. L. *Advance in Agronomy*. Vol. IV, 1952.
- Nicholas, D. S. D. *J. Hort. Sci.*, **25** : 60-77, 1949.
- Ochoa, S. *J. Biol. Chem.*, **174** : 133-157, 1948.
- Rudra, M. N. *Nature*, **144** : 868, 1939.
- Samuel, G. and Piper, C. S. *Ann. Applied Biol.*, **16** : 493-524, 1929.
- Shive, J. W. *Plant Physiol.*, **16** : 435-445, 1941.
- Singh, A. and Pal Archana. *Proc. Nat. Acad. Sci. Sec. B.*, vol. XXXIII part II, pp. 216-226, 1963.
- Smith, E. L. See Edsall J. J., *Enzymes and Enzyme systems*. Harbard. Univ. Press, Cambridge, 1951.
- Sommers, I. I. and Shive, J. L. *Plant Physiol.*, **17** : 582, 1942.
- Steinberg, R. A. See Emil Troug's mineral nutrition of plants. The University of Wisconsin Press, 358-386 pp., 1953.

# SOME PHYSIOLOGICAL STUDIES ON *PESTALOTIOPSIS* *VERSICOLOR* (SPEG.) STEY.

By

R. N. TANDON and S. K. MITRA

Botany Department, University of Allahabad

[Received on August 1, 1962]

Species of the genus *Pestalotiopsis* causing leaf spot and fruit rot diseases are of common occurrence throughout the world, but so far detailed physiological investigations have been carried out on a few of them only.

Tandon and Bilgrami (1957) working with *Pestalotia mangiferae* and Tandon and Bhargava (1960) on *Pestalotia* sp. reported that their organisms showed maximum growth on slightly acidic medium (*i.e.* on pH 5.6 to 6.5). Ito *et al.* (1954) as well as Tandon and Bhargava (1960) found that the best growth and sporulation of *Pestalotia* sp. isolated from *Camellia japonica* and *Livistona rotundifolia* were within a temperature range of 19° to 25°C.

A scrutiny of the available literature shows that different sources of carbon, nitrogen, sulphur and phosphorus do not support maximum growth and sporulation of every organism. In view of the above fact it was considered desirable to carry out detailed physiological studies on *Pestalotiopsis versicolor* isolated from the diseased leaves of *Eugenia jambolana* to find out the most suitable sources of these essential constituents of a synthetic medium for its maximum growth and sporulation.

## Materials and Methods:

*Pestalotiopsis versicolor* (Speg.) Stey., was isolated from the diseased leaves of *Eugenia jambolana*. Single spore cultures were prepared by the usual method. Asthana and Hawker's medium A\* was used as a basal medium for maintaining the stock cultures and for adjusting the various constituents for detailed study.

150 c.c. Pyrex conical flasks and guaranteed pure reagents were used throughout the investigation. 25 c.c. of liquid media were autoclaved at 15 lbs. pressure for 15 minutes. Fractional sterilization was carried out whenever complex carbohydrates or compounds containing organic nitrogen were used in the culture medium. In every experiment the organism was allowed to grow undisturbed for 15 days. The fungal mat was then filtered on previously weighed oven dried Whatman's filter paper No. 42. The filter papers containing the fungal mats were kept in an electric oven maintained at 70°C for 2 days, cooled in a desiccator and reweighed to four places of decimal. The difference between the final and initial weights gave the dry weight. In each experiment three replicates were taken. The dry weight results were statistically analysed wherever necessary. In all experiments dealing with nutritional studies, care was taken to supply the same amounts of carbon, nitrogen, sulphur and phosphorus as were available in the basal medium. Wherever the formulae of carbon or nitrogen compounds were not definite, the amount added was equal to the weight of carbon or nitrogen source present in the basal medium.

\*Glucose 5.0 g., KNO<sub>3</sub> 3.5 g., KH<sub>2</sub>PO<sub>4</sub> 1.75 g., MgSO<sub>4</sub> 7H<sub>2</sub>O 0.75 g., and distilled water 1000 ml.



The acidity or alkalinity of the medium was modified with the help of hydrochloric acid or sodium hydroxide and B.D.H. indicator papers. The degree of sporulation was measured on the basis of pseudopycnidial masses developed as well as on the number of spores present in the slides examined microscopically. General mean  $\pm$  C.D. (critical difference) was taken to be moderate and greater or lower dry weights than this limit were considered good and poor respectively.

### Observations :

#### (i) Effect of different hydrogen ion-concentrations :

The effect of different hydrogen ion-concentrations on the growth and sporulation of the present organism was studied. The initial pH of the medium was varied from pH 2.5 to 10.0 and the following values were adjusted—2.5, 3.4, 4.3, 5.2, 5.9, 6.4, 7.0, 7.5, 8.0, 9.0 and 10.0. The results are summarized in table 1.

TABLE 1  
Showing the dry weight and sporulation of *Pestalotiopsis versicolor*  
at different pH

pH of the medium	Dry weight in mg	Sporulation
2.5	—	—
3.4	55.8	Poor
4.3	62.8	Good
5.2	72.5	Excellent
5.9	80.2	Good
6.4	70.1	Good
7.0	61.8	Fair
7.5	59.9	Poor
8.0	55.5	Poor
9.0	51.3	Poor
10.0	44.4	—
General mean = 61.7 mg		

Summary of the dry weight results and conclusions at 5% level of probability are given below :

Treatments	.. Highly significant
Replicates	.. Insignificant
Standard error	.. 5.13
Critical difference	.. $\pm$ 10.77

The different treatments can be grouped as follows :

Good	= pH 5.9 and 5.2
Moderate	= pH 3.4, 4.3, 6.4, 7.0, 7.5 and 8.0
Poor	= pH 9.0 and 10.0

The sporulation was best at pH 5.2 and good at pH 4.3, 5.9 and 6.4. It was, however, poor at both the extremes of acidic and alkaline side. The fungus was incapable of growing at pH 2.5. In general, it was observed that with an increase in the pH of the medium there was a tendency towards the suppression of the aerial mycelium.

(ii) *Effect of temperature :*

In order to study the effect of different temperatures on the growth and sporulation of the present organism, it was inoculated and then incubated at 5°C, 10°C, 22 ± 2°C (room temperature), 25°C, 30 ± 2°C and 35 ± 2°C. It was observed that the fungus was incapable of growing at 5°C and 35 ± 2°C. Best growth and sporulation of the organism was obtained at 22 ± 2°C and 25°C. The growth was poor at 10°C and 30 ± 2°C. The sporulation was poor at 30 ± 2°C and absent at 10°C.

(iii) *Effect of different sources of carbon :*

As the basal medium contained 2.0 g of carbon per litre, it was decided to supply the same amount of carbon from the following compounds, viz., xylose, sorbose, glucose, galactose, fructose, sucrose, lactose, maltose, raffinose, starch, glycerol, mannitol, sorbitol, malic and tartaric acid.

The dry weight sporulation and other microscopic characters of the organism on different carbon sources are recorded in table 2.

TABLE 2  
Showing the dry weight, sporulation, and other microscopic characters  
of *Pestalotiopsis versicolor* on different sources of carbon

Sources of carbon	Dry weight in mg	Degree of sporulation	Thickness of hyphae in $\mu$	Size of spores in $\mu$	Size of Pseudopycnidia in $\mu$
Xylose	—	—	—	—	—
Sorbose	34.4	—	1.87	—	—
Glucose	63.2	Good	2.05	15.35 × 5.15	172 × 140
Galactose	36.4	Poor	2.05	13.42 × 5.30	—
Fructose	49.1	Excellent	2.42	16.50 × 5.07	—
Sucrose	53.6	Good	2.42	15.37 × 5.0	—
Lactose	19.8	—	2.0	—	—
Maltose	73.4	Excellent	1.87	13.2 × 5.17	116 × 88
Raffinose	59.6	Good	2.40	15.50 × 5.05	—
Starch	68.2	Excellent	2.57	15.42 × 5.12	128 × 108
Glycerol	—	—	—	—	—
Sorbitol	30.6	—	2.12	—	—
Mannitol	46.7	Good	2.42	16.42 × 5.02	116 × 92
Malic acid	—	—	—	—	—
Tartaric acid	29.4	Good	2.12	14.17 × 5.0	—
No carbon source	—	—	—	—	—
General mean = 47.0 mg					

Summary of the dry weight results and conclusions at 5% level of probability are as follows :

Treatments	.. Highly significant
Replicate	.. Insignificant
Standard error	.. 1.67
Critical difference	.. $\pm 3.4$

The different treatments can be grouped as follows :

- Good = maltose, starch, glucose, raffinose and sucrose.
- Moderate = fructose and mannitol.
- Poor = galactose, sorbose, sorbitol, tartaric acid and lactose.

The above results indicate that the fungus under investigation grew best on carbon sources which yielded glucose on hydrolysis (*viz.* maltose and starch), but the hyphae were thinnest on maltose and sorbose. The size of the spores was slightly bigger on fructose and mannitol and smallest on maltose. It is thus obvious that there was no correlation between the best growth, sporulation, size of spores and the thickness of the hyphae of the present organism.

The effect of different concentrations of glucose was also studied. It was noticed that the dry weight of the fungus continued to increase with an increase in the amount of glucose from 0.5 g to 50 g per litre. The fungus could not grow in the absence of glucose. Higher concentrations of glucose (*viz.* 30, 40 and 50 g per litre), decreased the sporulation. It was best when 7.0 to 25 g per litre of glucose was supplied in the medium.

(iv) *Effect of different sources of nitrogen :*

The following different sources of nitrogen *viz.* potassium nitrate, ammonium nitrate, ammonium chloride, ammonium sulphate, magnesium nitrate, sodium nitrate, sodium nitrite, urea, thiourea, asparagine, peptone, glycine and glutamic acid were added singly in the basal medium in place of the usual nitrogen source. The quantity of nitrogen taken was 485 mg of nitrogen per litre and thus it was the same as in the basal medium. The dry weight and other microscopic characters are recorded in table 3.

From table 3, it is obvious that the present fungus grew best on organic sources of nitrogen (*viz.* peptone and asparagine). Magnesium nitrate was the best inorganic source of nitrogen. The sporulation was best on peptone, ammonium chloride and potassium nitrate. It was fair only on asparagine. It is thus clear that in this case also there was no correlation between the best growth and best sporulation. The size of the spores did not show any marked effect except that they were a bit smaller on ammonium chloride and asparagine than on sodium nitrate.

The effect of different concentrations of potassium nitrate was also studied. Increased growth of the fungus was observed upto 4.0 g. per litre, thereafter any further increase decreased the growth. The sporulation was only fair at lower and higher concentrations of  $\text{KNO}_3$  *viz.* 0.5 to 2 g and 20 to 30 g per litre. It was good when 3.0 to 14.0 g of  $\text{KNO}_3$  per litre was supplied.

(v) *Effect of different sulphur sources :*

The following sulphur compounds *viz.* potassium sulphate, magnesium sulphate, potassium meta-bi-sulphite, sodium hyposulphite, sodium thiosulphate,

thiourea and methionine were added singly as a sulphur source in the basal medium. The amount of sulphur in each case was 98 mg per litre and was thus similar to that present in 0.75 g of magnesium sulphate. The dry weight and other microscopic characters are recorded in table 4.

TABLE 3

Showing the dry weight, sporulation and other microscopic characters of *Pestalotiopsis versicolor* on different sources of nitrogen

Sources of nitrogen	Dry wt. in mg	Degree of sporulation	Size of spores in $\mu$	Size of pseudopycnidia in $\mu$
Potassium nitrate	65.2	Excellent	15.50 $\times$ 5.0	125.0 $\times$ 67.5
Ammonium nitrate	52.3	Good	16.0 $\times$ 4.82	
Ammonium chloride	67.2	Excellent	14.80 $\times$ 5.0	
Ammonium sulphate	64.4	Good	16.07 $\times$ 4.92	
Magnesium nitrate	70.9	Good	15.75 $\times$ 4.92	130.0 $\times$ 67.5
Sodium nitrate	53.1	Good	17.5 $\times$ 4.87	
Sodium nitrite	25.4	—	—	
Urea	60.6	Good	15.0 $\times$ 5.0	
Thiourea	11.5	—	—	130.0 $\times$ 67.5
Asparagine	77.2	Fair	14.5 $\times$ 5.0	
Peptone	85.2	Excellent	16.0 $\times$ 4.87	
Glycine	59.4	Good	16.5 $\times$ 5.0	
Glutamic acid	67.7	Good	15.85 $\times$ 4.85	
No nitrogen source	—	—	—	
General mean	= 58.4 mg			

Summary of the dry weight results and conclusions at 5% level of probability are given below:

Treatments	.. Highly significant
Replicates	.. Insignificant
Standard error	.. 1.0
Critical difference	.. $\pm 2.06$

The different treatments can be arranged as follows:

Good = peptone, asparagine, magnesium nitrate, glutamic acid, ammonium chloride, potassium nitrate, ammonium sulphate and urea.

Moderate = glycine.

Poor = sodium nitrate, sodium nitrite and thiourea.

TABLE 4

Showing the dry weight, sporulation and other microscopic characters of *Pestalotiopsis versicolor* on different sources of sulphur

Sources of sulphur	Dry wt. in mg.	Degree of sporulation	Size of spores in $\mu$	Size of chlamydo-spore in $\mu$	Range in the size of pseudopycnidia in $\mu$
Potassium sulphate	65.4	Good	14.5 × 5.0	—	56 × 48
Magnesium sulphate	64.8	Good	14.6 × 5.0	—	—
Potassium meta-bi-sulphite	49.8	Fair	15.0 × 5.0	2.5 × 5.0	40 × 40
Sodium hyposulphite	44.8	Good	16.0 × 4.9	—	80 × 100–132 × 108
Sodium thiosulphate	51.8	Good	16.0 × 5.0	—	36 × 36–92 × 60
Thiourea	30.6	Good	15.0 × 5.0	—	188 × 200
Methionine	48.0	—	—	3.75 × 5.0 to 12.5 × 10.0	—
No sulphur source	25.2	—	—	—	—
General mean = 47.5 mg					

Summary of the dry weight results and conclusions at 5% level of probability are given below :

Treatments	.. Highly significant
Replicates	.. Insignificant
Standard error	.. 2.23
Critical difference	.. $\pm 4.77$

The treatments can be grouped as follows :

Good = potassium sulphate and magnesium sulphate.

Moderate = potassium meta-bi-sulphite, sodium thiosulphate, sodium hyposulphite and methionine.

Poor = thiourea.

Table 4 shows that the growth of *Pestalotiopsis versicolor* was best on potassium and magnesium sulphate. Sporulation was, however, good on all the sources of sulphur tried except on potassium meta-bi-sulphite and methionine (where it could not sporulate). There was no marked change in the size of the spores except that they were slightly smaller on potassium and magnesium sulphate.

The effect of different concentrations of magnesium sulphate was also studied. Increase in its concentration upto 2.0 g per litre showed gradual increase in growth but any further increase in its quantity decreased the growth. The sporulation was good on 1 to 3.0 g per litre of magnesium sulphate.

Among the four different phosphorus sources tried *viz.* potassium tri-basic phosphate, potassium di-hydrogen phosphate, potassium di-basic phosphate, and sodium phosphate, the former two were observed to support the best growth of

the present fungus. The other two supported moderate growth only but the sporulation was excellent.

The study on the effect of different concentrations of potassium di-hydrogen phosphate revealed that the growth of the fungus increased upto 3.0 g of  $\text{KH}_2\text{PO}_4$  per litre. Any further increase decreased the growth. The sporulation was best between 2.0 to 3.0 g per litre of  $\text{KH}_2\text{PO}_4$ .

#### Discussion :

The present organism was capable of growing within a wide range of pH (*viz.* 3.4 to 10.0). It failed to grow at pH 2.5. In contrast to this Tandon and Bhargava (1960) had reported that *Pestalotia* sp. on *Livistona rotundifolia* could grow even at pH 1.5.

The fungus under study could grow only within a temperature range of  $10^\circ\text{C}$  to  $30 \pm 2^\circ\text{C}$ . The growth and sporulation were, however, best between  $22^\circ\text{C}$  to  $25^\circ\text{C}$ . Similar results were obtained by Ito *et al.* (1954) and Tandon and Bhargava (1960) working with different species of *Pestalotia*.

Studies on the effect of different carbon sources revealed that maltose, starch and glucose were the best sources of carbon for the growth and sporulation of the present organism. Tandon (1950) found maltose to be the best source of carbon for the growth of *Pestalotia malorum*. Thind and Randhawa (1957) also obtained good growth of *Colletotrichum capsici* on this sugar. Raffinose and sucrose were also found to be statistically good sources of carbon for the growth of the present species of *Pestalotiopsis*. Working with *Pestalotia* sp. Tandon and Bhargava (1960) reported that the growth was good on glucose and best on sucrose.

Increase in the amount of glucose increased the growth of the fungus even upto a concentration of 50 g per litre. The sporulation also increased with an increase in the amount of glucose upto a certain limit (*viz.* 25 g per litre). Bilgrami (1956), Verma (1957) and Tandon and Bhargava (1960) made similar observations for *Pestalotia mangiferae*, *Pestalotia* sp. on *Mimusops elangi* and *Pestalotia* sp. on *Livistona rotundifolia* respectively.

Peptone and asparagine were the best sources of nitrogen for the present organism. Tandon (1950) working with *Pestalotia malorum* and *Pestalotia psidii* also observed that the best growth of those organisms was on peptone. Tandon and Bhargava (1960) observed asparagine to be the best source of nitrogen for *Pestalotia* sp. It was, however, a moderate source of nitrogen for this fungus. Glutamic acid, ammonium chloride, potassium nitrate and ammonium sulphate were found to be statistically good sources of nitrogen for the present organism.

Though the different carbon and nitrogen sources influenced the sporulation of the fungus but there was no correlation with growth. Fergus (1952), Hacskeyalo *et al.* (1954), Agarwal (1955), Bilgrami (1956) also obtained similar results with the organisms studied by them. The growth increased upto 4 g of  $\text{KNO}_3$  per litre and it decreased with any further increase of this substance.

Potassium sulphate and magnesium sulphate were found to be the best sources of sulphur for the growth and sporulation of the organism under investigation. Some growth was observed even in the absence of any sulphur. Increase in the concentration of magnesium sulphate upto 2.0 g per litre showed gradual increase in the amount of growth. The sporulation was, however, best between 1 to 3 g per litre of magnesium sulphate.

Potassium tri-basic phosphate and potassium di-hydrogen phosphate were the best sources of phosphorus for the growth and sporulation of the present organism.

Tandon (1950), Verma (1957) and Tandon and Bhargava (1960) working with different species of *Pestalotia* obtained similar results. The growth of the fungus increased upto 3.0 g per litre of  $\text{KH}_2\text{PO}_4$  and any further increase in its concentration adversely influenced the growth.

#### Summary :

An attempt was made to study the physiology of *Pestalotiopsis versicolor* causing leaf spot disease of *Eugenia jambolana*.

The fungus could grow within a pH range of 3.4 to 10.0, but it could not grow at pH 2.5. Best growth of the fungus was observed at a temperature of 22°C to 25°C. The present organism showed an increase in the growth upto 50 g. of glucose per litre but the sporulation decreased at higher as well as at very low concentrations of glucose (*viz.* below 3.0 and above 14.0 g per litre). Maltose was found to be the best source of carbon.

The best sources of nitrogen were found to be peptone and asparagine. The growth increased upto 4.0 g of  $\text{KNO}_3$  per litre, but any further increase in its concentration had adverse effect.

Potassium sulphate and magnesium sulphate were the best sources of sulphur. The growth increased with an increase in the concentration of magnesium sulphate upto 2.0 g per litre, further increase in the amount of magnesium sulphate, however, decreased the dry weight.

Potassium tri-basic phosphate and potassium di hydrogen phosphate were good sources of phosphorus. The growth of the fungus increased upto 3.0 g of  $\text{KH}_2\text{PO}_4$  per litre and any subsequent increase resulted in decreased growth.

#### Acknowledgements :

The authors are thankful to Dr. K. S. Bilgrami for supplying the culture of the pathogen and for ungrudging help at various stages. The junior author is also thankful to the University of Allahabad for the award of the Departmental Scholarship.

#### References :

- Agarwal, G. P. *D.Phil. Thesis*, University of Allahabad, 1955.  
Bilgrami K. S. *Ibid*, 1956.  
Fergus, C. L. The nutrition of *Penicillium digitatum*. *Mycologia*, **44** : 183-199, 1952.  
HacsKayalo, J., Lilly, V. G. and Barnett, H. L. Growth of fungi on three sources of nitrogen. *Mycologia*, **46** : 691-701, 1954.  
\*Ito, K., *et al.* *Bull. Expt. Station Mergu*, 103-128. 1954.  
Tandon, M. P. *D.Phil. Thesis*, University of Allahabad, 1950.  
Tandon, R. N. and Bilgrami, K. S. Cultural studies of *Pestalotia magniferae* *Proc. Nat. Acad. Sci., (India)* **27B** : 98-105, 1957.  
Tandon, R. N. and Bhargava, S. N. Some physiological studies of *Pestalotia* sp. causing leaf spot disease of *Livistona rotundifolia*. *Proc. Nat. Acad. Sci., (India)* **30B** : 257-65, 1960.  
Thind, K. S. and Randhawa, H. S. Studies on nutrition of fungi I. The influence of different carbon compounds on the growth and sporulation of *Colletotrichum capsici* (Syd.) Butler and Bisby. *Proc. Nat. Acad. Sci., (India)* **27B** : 39-46, 1957.  
Verma, S. N. P. *M.Sc. Thesis*, University of Allahabad, 1957.

\*Original not consulted.

MALE REPRODUCTIVE ORGANS OF *LATHRECISTA ASIATICA*  
*ASIATICA* FABRICIUS

(LIBELLULIDAE : ODONATA)

By

BRIJESH KUMAR SRIVASTAVA

*Department of Zoology, University of Saugar, Saugar, India*

[Received on December 12, 1962]

Selys-Longchamps and Hagen (1958), Palmen (1884), Goddard (1896), Thompson (1908) and Machotin (1929) were among the early workers who investigated the external genital organs of the male Odonata. Marshall (1914) described the anatomy of the reproductive organs in *Libellula quadrimaculata* Linn. George (1928) gave a brief account of the morphology and development of the genital organs in Odonata. The best general works on the reproductive organs of male dragonflies are perhaps those of Tillyard (1917), Grasse (1949) and Tuxen (1956).

**Material and Method**

The adults of *Lathrecista asiatica asiatica* Fabr. were captured with a large net measuring 42 inches in length and 24 inches in diameter, during the post-monsoon period (i.e. from middle of September to November). For histological studies, the dragonflies were pinned alive in a small dissecting dish and dissected in 0.75% NaCl solution. After-dissection the salt solution was replaced by a mixture of Picro-Formol-Acetic Acid in the ratio 3 : 1 : 0.2 and the reproductive organs fixed for 18-20 hours in it. Serial sections, 6-8 micra thick, were stained either with Delafield's haematoxylin or Heidenhain's haematoxylin and counter-stained with Eosin or Orange G. Whole mounts of the internal genital organs were fixed in ordinary Bouin's fluid, stained in Borax carmine and mounted in thick Canada balsam after going through the usual procedure of washing and dehydration.

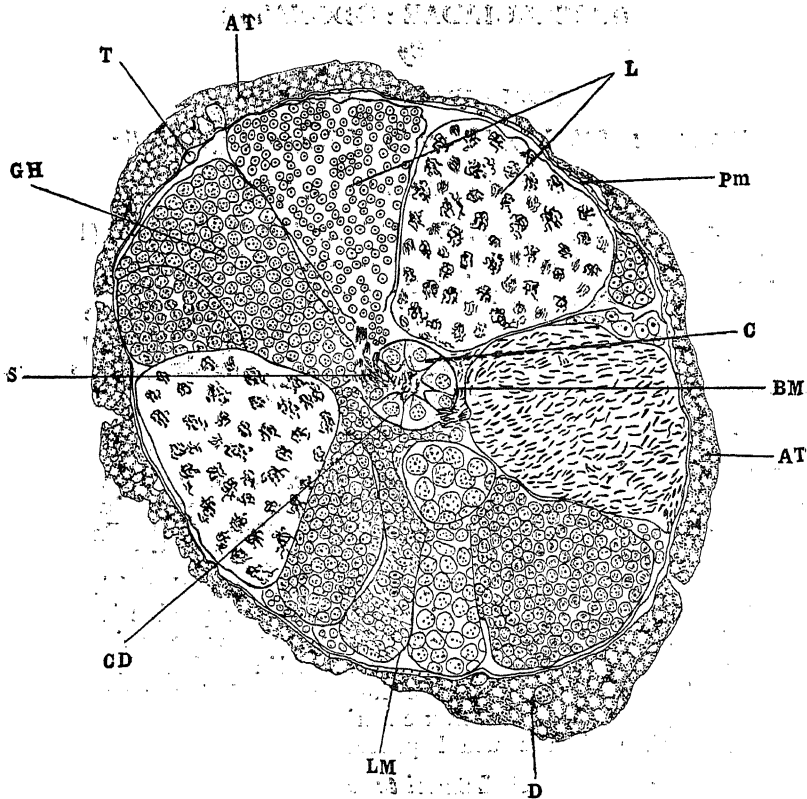
Male reproductive organs of *Lathrecista asiatica asiatica* Fabr. consist of a pair of testes, a pair of ducts, the vasa deferentia, a common median sperm-sac (constituting the internal genital organs) and external genital organs.

**Internal Genital Organs**

**(A) Gross Morphology :**

*The Testes.*—The testes are a pair of long, tubular, almost translucent and multi-lobular organs (Plate III, fig. 5) lying ventro-laterally to the alimentary canal one on either side. Each testis is 5.7 mms. long and 0.336 mm. thick and extends from the posterior half of the sixth abdominal segment upto the end of the seventh abdominal segment (Plate I, fig. 1). The testes may be larger and may occupy more space or the position of the two testes may be different. In the latter case one may be a little anterior or posterior to the other. The testis is maintained in its position by tracheae, nerves, fat-bodies and also by filaments, being the anterior prolongations of the adipose tissue surrounding the testis. These filaments are attached to the body wall in the fifth abdominal segment.





Text figure 1. Camera lucida sketch of cross section of the testis.

AT = Adipose tissue

BM = Basement membrane

C = Epithelial lining

CD = Central duct

D = Nucleus

GH = Germ cells

L = Lobules

LM = Limiting membrane of the lobule

Pm = Peritoneum

S = Spermatozoa

T = Tracheole

**The Vas Deferens.**—Each testis is continued posteriorly into a duct, the vas deferens, which is marked off from the former by a prominent constriction. The vas deferens is a slender whitish tube extending from the beginning of the eighth abdominal segment up to the middle of the ninth abdominal segment (Plate I, fig. 1) and like the testis, lies ventro-laterally to the alimentary canal. The vas deferens is nearly as long as the testis. Its average length in the living insect is 4.8 mms. while its thickness measured in the fixed preparations is 0.16 mm. Except at the constriction the diameter of the vas deferens is more or less uniform. The vas deferens does not form any loop before joining the sperm-sac, a fact, contrary to the observations of the author (1960) in *Bradinopyga geminata* Rambur.

**The Sperm-sac.**—The vasa deferentia of the two sides unite posteriorly to form a very small dilated common duct, just above the male gonopore, forming a white sac-like structure, the sperm-sac or the common vesicula seminalis (Plate I, fig. 1). The two vesiculae seminales are incompletely separated from each other by a dorsal ridge which is indicated externally by a mid-dorsal notch (Plate I, fig. 3). The sperm-sac is more or less oval in structure measuring 0.496 mm. in antero-posterior direction and 0.64 mm. across, and lying ventrally to the hind-gut. A portion of the ventral wall of the sperm-sac lies closely applied to the inner sides of a chitinous cup-like pit, formed by the invagination of the ventral body wall above the male gonopore.

**The Ejaculatory Duct.**—The sperm-sac communicates directly to the exterior on the ventral side, through a very short tube, the ejaculatory duct (Plate I, fig. 3) which fits into the cup-like invagination (r) (Plate III, fig. 6) of the ventral chitinous body wall. It opens out through the male genital opening by an elliptical aperture.

**The Accessory Glands.**—Accessory glands are totally absent in male *L. asiatica* Fabr.

#### (B) Histology :

**The Testes.**—The wall of the testis (Text figure 1) consists of an outer layer of adipose tissue followed by an inner peritoneal layer. The adipose tissue forms a complete and regular layer around the testis and is very closely applied to the peritoneum surrounding the organ. It consists of a very irregular, spongy and highly vacuolated mass of large cells with big and granular nuclei. Fat bodies form the greater bulk of this tissue and are very prominent. The peritoneal layer is the only limiting layer of the testis and covers all the lobules. Peritoneum is represented by a single layer of very thin, stretched cells with comparatively big and somewhat elongated nuclei. Because it is the investing layer of the testis, it may be termed the 'scrotum' after Imms (1957).

Internally each testis comprises a very large number of more or less spherical testicular lobules attached to a central duct running straight throughout the length of the testis. The wall of the central duct consists of a single tier of large cuboidal cells forming the epithelium. The epithelial cells contain large nuclei and rest on a basement membrane. The lumen of the duct contains sperms.

**Structure of Testicular Follicle or Lobule.**—A lobule of the dragonfly testis is a solid, almost spherical mass, composed of a large number of germ cells surrounded by a thin wall. The size of the lobules varies greatly. Externally each testicular lobule is surrounded by a thin limiting membrane. The interior of the lobule is packed with germ cells leaving no space in centre. All the germ cells in a single lobule are similar and represent only one definite stage of spermatogenesis. It means that the germ cells in successive stages of spermatogenesis i.e. spermatogonia, spermatocytes, spermatids and spermatozoa do not occur together in one lobule as

is found in the testicular lobules of most insects. The posterior end of each lobule forms a very small tube which opens into the central duct measuring 0.032 mm. in diameter. The efferent duct of the lobule is very small and is seemingly the prolongation of the lobule itself. As such it has the same structure as the lobule. It is interesting to note the absence of a germinal epithelium in the lobule of an adult dragonfly testis.

*The Vas Deferens.*—It is a very simple tube of an uniform structure throughout and is made up of four layers from within outwards (Plate I, fig. 2) : (i) Epithelial layer, (ii) Basement membrane, (iii) Musculature and (iv) Layer of fat bodies.

The epithelial layer forms the innermost wall of the duct. It consists of a single layer of cuboidal cells which are provided with distinct large, somewhat ovoid nuclei. There is a thin structureless basement membrane which supports the basal ends of the epithelial cells. A thin layer of circularly disposed muscle fibres surrounds the basement membrane. The outermost investment is formed by a layer of adipose tissue, which is continuous with that surrounding the testis. In structure it is identical to the adipose tissue of the testis. The central lumen of the vas deferens contains sperms and is devoid of a chitinous lining. The vas deferens towards the posterior region shows a somewhat different structure. The epithelial cells are columnar and musculature is well developed in this region. On the whole the wall of the vas deferens is comparatively thicker posteriorly showing, however, essentially the same structure as the anterior region.

*The Sperm-sac.*—Posteriorly the vasa deferentia swell up to form two vesiculæ seminales, which unite in the middle of the ninth abdominal segment (Plate I, fig. 3). Both the seminal vesicles are enclosed in a common muscular coat which in itself is surrounded by the layer of adipose tissue. The point where the two vasa deferentia unite is marked by a very prominent dorsal ridge, projecting into the cavity of the sac in the sagittal plane. This ridge is in the form of a semi-circular arc on the dorsal surface. Histologically the cells lining the ridge are quite different from the neighbouring epithelial cells and consist of long columnar gland cells with granular nuclei and vacuolated cytoplasm. The epithelium of the wall of the seminal vesicle consists of a single row of columnar cells with big nuclei and is devoid of a chitinous lining. The lumen of the sperm-sac shows a very large number of irregularly scattered spermatozoa.

*The Ejaculatory Duct.*—The ejaculatory duct shows a structure similar to that of the seminal vesicle but the cells lining this tube possess highly granular nuclei (suggestive of their secretory nature). In the anterior region, the epithelial layer of the duct shows somewhat more or less branched cells. The lumen of the duct is lined by a thin chitinous intima.

### External Genital Organs

The male external genitalia in *L. asiatica asiatica* Fabr. consists of a pair of supra-anal appendages, an unpaired infra-anal appendage, a pair of small appendages surrounding the male gonopore and a complicated but characteristic secondary copulatory apparatus situated on the ventral side of the second and a part of the third abdominal segments.

*The Supra-anal Appendages.*—There is a pair of characteristically well developed dorsal supra-anal appendages arising from the last visible abdominal segment (Plate II, figs. 1 and 2) ; each measuring 2.1 mms. in length, approximately twice the length of the anal appendages of the female dragonfly. The supra-anal appendages are hollow cylindrical structures almost parallel at the bases but diverging apart from each other distally. Their distal ends are swollen before

terminating in a very small 0.425 mm. long dark spine. Each appendage bears laterally, towards the mid-dorsal line, a small spur-like process at the proximal end. All over the surface of the supra-anal appendage are found numerous bristle-like hairs, each situated on a small protruberance. The spine area is devoid of hair. Hairs are, however, short in the area facing the infra-anal appendage. 4-6 black small teeth-like protruberances (Plate II, figs. 1 and 3) arranged in a row are found on the ventro-lateral margin of each supra-anal appendage towards the distal end. The supra-anal appendages are uniformly coloured black.

*The Infra-anal Appendage.*—The infra-anal appendage or appendix dorsalis (Tuxen 1956) is apparently a single spatula-shaped triangular piece, slightly smaller in size than the supra-anal appendage and lies ventral to it but dorsal to the anus (Plate II, figs. 1, 2 and 3). The tip is slightly rounded, blackish-brown in colour, and bears two black tubercles arranged symmetrically on the two sides of a faintly indicated middle suture. The surface of the infra-anal appendage bears bristle-like hairs. The basal area of the appendage on the ventral side is grooved in order to receive the supra-anal lamina (Lamina analis), which borders the anus. The infra-anal appendage is present only in the males and is coloured black dorsally and ventrally, except near the base which is dark pale-brownish in colour.

*The Male Gonopore.*—The male genital opening or genital meatus (Tuxen, 1956), is situated ventrally in the middle of the ninth abdominal segment (Plate II, fig. 3) and (Plate III, fig. 6). It is a somewhat elongated elliptical orifice measuring 0.153 mm. in length, and is guarded by two strongly sclerotized pieces, the gonopods. The gonopods are thick and pear-shaped appendages measuring 0.561 mm. in length and are present in the middle of the ninth abdominal segment on the ventral side. The tapering ends of these appendages are directed posteriorly. The sternum of the ninth segment becomes modified to form an anterior genital plate and a post-genital plate lying respectively anterior and posterior to the gonopore. The anterior genital plate measures 0.833 mm. in length and is densely beset with numerous hairs and minute tubercles. The post-genital plate is a small oval structure bearing numerous long hairs.

The chitinous wall of the male genital aperture is drawn up dorsally to form a small heart-shaped structure (*r*) (Plate III, fig. 6), measuring 0.391 mm. long and 0.255 mm. wide, into which fits the ejaculatory duct. Ventrally the cup bears few scattered hairs on the lateral side.

*The Secondary Copulatory Apparatus.*—It consists of genital lobes, anterior lamina, posterior lamina, genital fossa, lateral supporting framework, a pair of hamules, penis sheath, penis vesicle and an intromittent organ (Plate II, fig. 5; Plate III, figs. 1 and 2). Penis and the penis vesicle are developed from the anterior part of the third sternite (Thompson, 1908). Of these, the vesicle remains in the third segment, but the penis moves forward to lie in the second abdominal segment.

*The Genital Lobes.*—The tergum of the second abdominal segment gives out from its postero-lateral sides a pair of latero-ventral processes, the genital lobes, which lie almost at right angles to the plane of abdomen and measure 0.578 mm. in length. The genital lobes are club-like, slightly constricted at the base, but are roundly expanded at the apex and overlap the middle part of the vesicles of the penis and the posterior ends of the hamules. The dorsal surface and the margin of the lobe bear bristle-like hairs which are thickly set on the ventral apical end of the lobe. Fraser (1936) has not shown hairs on the genital lobes in the "Fauna of British India".

*The Genital Fossa.*—All the secondary copulatory organs are lodged in a membranous depression, the genital fossa or fenestra (Tuxen, 1956), formed on the ventral side of the second abdominal segment. The genital fossa is bounded anteriorly by anterior lamina, posteriorly by posterior lamina and is strengthened laterally by the lateral supporting chitinous framework. The sheath of the penis lies immediately ventral to the fossa. The genital fossa has no connection with the cavity of the penis vesicle lying posteriorly, contrary to the observations of Tillyard (1917) and Imms (1957).

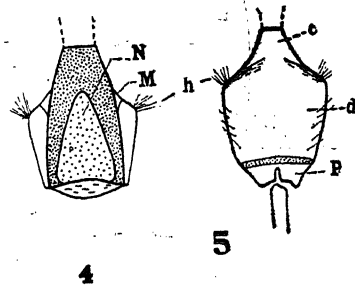
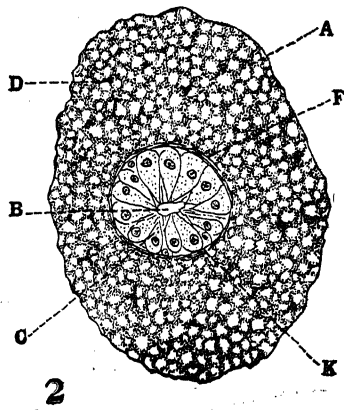
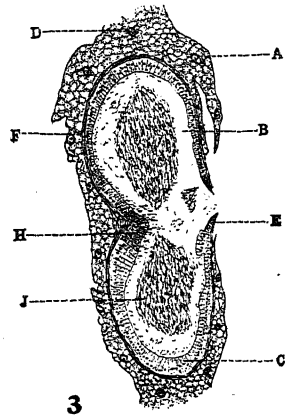
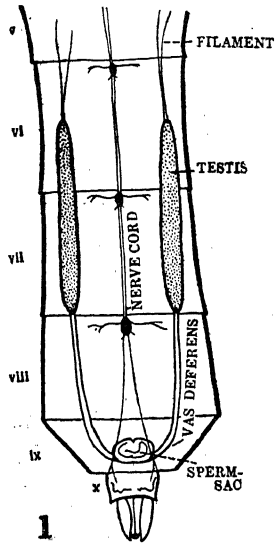
*The Anterior Lamina.*—It is a large chitinous piece lying ventro-anteriorly to the genital fossa and extending upto the middle of the second abdominal segment. This piece is divided into two regions; one small anterior (a), almost flat and broad plate, and the other large posterior (b), strongly sclerotized, convex hood-like plate. The anterior portion of the anterior lamina is weakly chitinized and bears many small tough hairs on its upper surface. The upper surface of the posterior plate of the anterior lamina bears numerous tubercles arranged in rows forming a V-shaped pattern. It also bears irregularly distributed minute hairs. Its posterior margin is wavy in outline without any indication of a median cleft and bears specially large stout hairs. The anterior lamina projects over the anterior rounded apical end of the penis sheath. The main function of the anterior lamina is to protect the secondary copulatory organs.

*The Posterior Lamina.*—It is a very weakly sclerotized sclerite, resembling the butterfly wings in shape, and is situated ventrally at the posterior end of the second abdominal segment, bordering the genital fossa posteriorly. Ventrally it is covered over by the hamules and the anterior region of the penis vesicle.

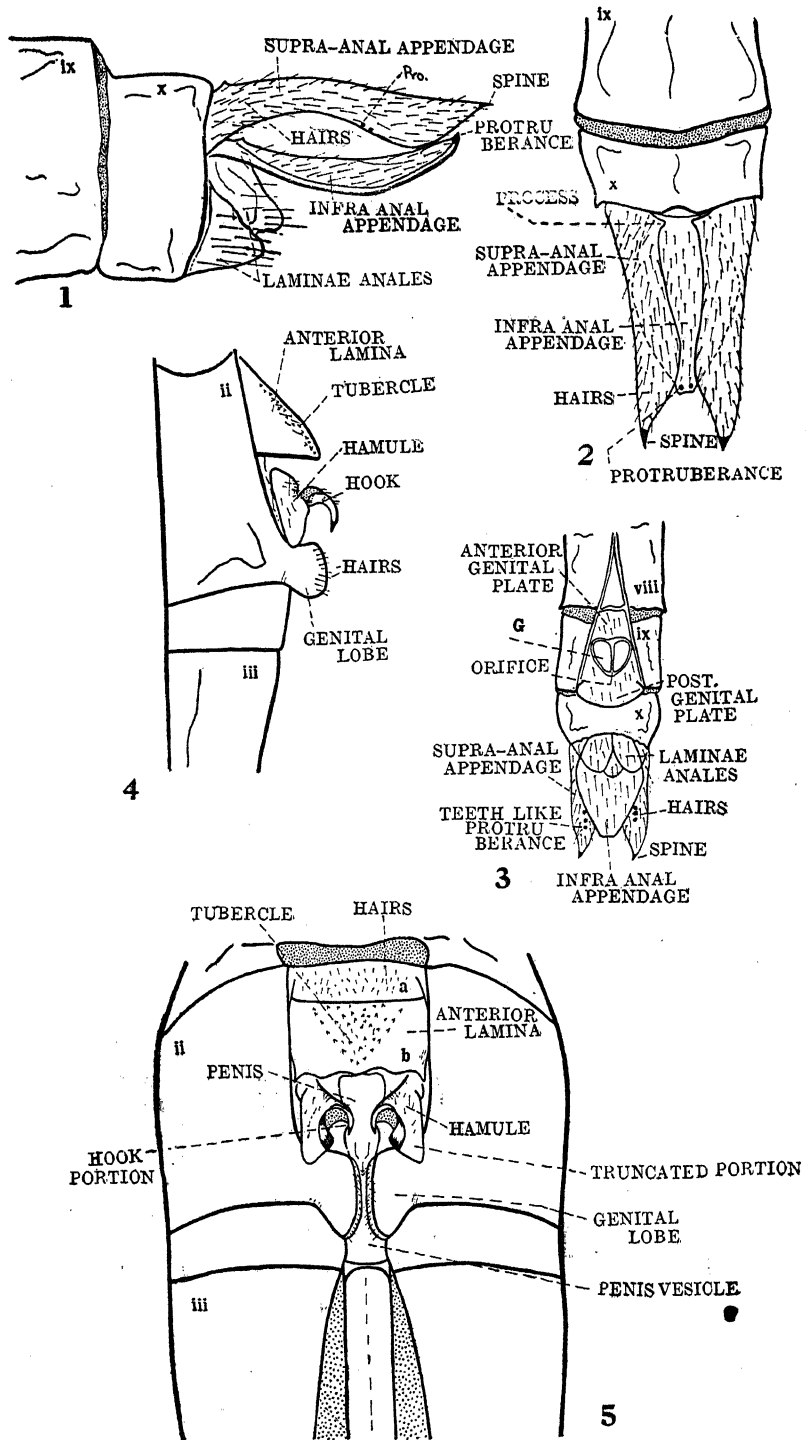
*The Supporting Framework.*—It is a system of skeletal chitinous rods forming roughly a U-shaped structure. These rods support the genital fossa laterally, the penis sheath dorsally and the anterior lamina anteriorly. The lateral elongated rods (z) (Plate III, fig. 2) measuring 0.935 mm. in length, are slightly convex ventrally and attached anteriorly to the underside of the antero-lateral basal ends of the posterior region of the anterior lamina. At their posterior ends, the lateral rods are joined by a dorsally curved rod which constitutes the horizontal bar (g) (Plate III, fig. 2) of the U-shaped framework. This structure gives support to the middle portion of the penis sheath from below. A distinct, short, elongated rod-like process arises ventro-laterally from about the middle of each of the lateral rods of the U-shaped framework, and provides the place for attachment of the anterior basal end of the hamule. The anterior ends of the lateral rods are weakly chitinized.

*The Penis Sheath.*—Arising from the posterior side of the genital fossa, and occupying more or less a central position on the ventral side of the second abdominal segment, is a scoop-like 0.816 mm. long structure, the penis sheath (Ligula of Tuxen, 1956) (Plate III, figs. 1 and 2) pointing anteriorly and bearing medially a broad shallow groove on the ventral side. The penis sheath is roughly triangular in shape and is divisible into two, almost equal, regions. One is the anterior triangular region (e) with a rounded apex and the other is a broad semi-circular postero-basal portion (f) which is more or less embedded in the membranous genital fossa. The anterior half region of the penis sheath is pale-yellow in colour due to the fact that it is comparatively more chitinized than the posterior half region. The dorsal surface of the penis sheath is supported in the middle by the median, dorsally curved rod of the framework and is distinctly sclerotized in the anterior one fourth portion. The posterior basal ends of the penis sheath are connected by a very faint line of chitin and give articulation to the posterior basal ends of the hamules.

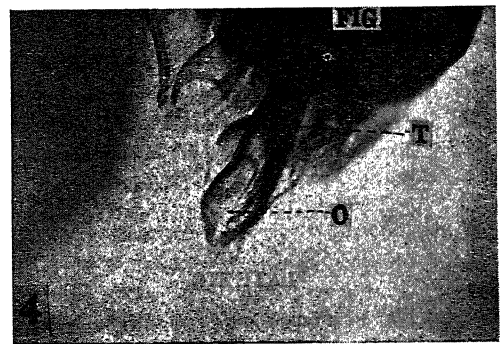
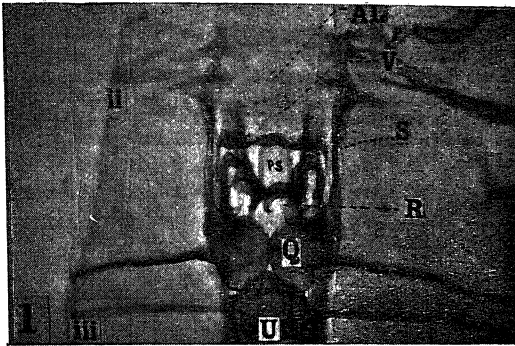
# PLATE I



# PLATE II



# PLATE III





## KEY TO LETTERING

A. Adipose tissue ; AL. Anterior lamina ; B. Lumen ; C. Epithelial lining ; c. Neck region ; D. Nucleus ; d. Posterior rectangular region ; E. Ejaculatory duct ; e. Apical region of penis sheath ; F. Musculature ; f. Basal region of penis sheath ; FIG. Figure of penis tip ; G. Gonopod ; g. Horizontal bar ; H. Dorsal ridge ; h. Hairs ; I. Proximo-basal end of hamule ; J. Spermatozoa ; K. Basement membrane ; k. Anterior genital plate ; L. Lobules ; l. Posterior genital plate ; M. Membranous area ; N. Dorsal chitinous sclerite ; O. Orifice ; P. Postero-medial sclerite ; PS. Penis sheath ; Pro. Protruberance ; Q. Genital lobe ; R. Hamule ; r. Cup-like chitinous structure ; S. Lateral supporting framework ; T. Tube ; U. Penis vesicle ; V. V-shaped pattern of tubercles on the anterior lamina ; W. Dorsal process ; X. Proximal end ; Y. Distal end ; Z. Lateral bar.

## EXPLANATION OF PLATES

### PLATE I. (All are Camera lucida diagrams).

- Fig. 1. Diagram of the dissection showing internal genital organs.  
 Fig. 2. T. S. of vas deferens (under high power).  
 Fig. 3. Section of sperm-sac (under low power).  
 Fig. 4. Penis vesicle (ventral view).  
 Fig. 5. Penis vesicle (dorsal view).

### PLATE II. (All are Camera lucida diagrams).

- Fig. 1. Lateral view of IX and X abdominal segments ( $2.0 \times 0.10 \times E$ ).  
 Fig. 2. Dorsal view of IX and X abdominal segments ( $1.5 \times 0.10 \times E$ ).  
 Fig. 3. Ventral view of abdominal segments VIII, IX and X ( $0.7 \times 0.10 \times E$ ).  
 Fig. 4. Lateral view of secondary copulatory apparatus ( $1.5 \times 0.10 \times E$ ).  
 Fig. 5. Ventral view of secondary copulatory apparatus ( $1.5 \times 0.10 \times E$ ).

### PLATE III.

- Fig. 1. Photomicrograph of secondary copulatory apparatus. Penis removed to show underlying organs (ventral view). ( $1 \times 0.6 \times E$ ).  
 Fig. 2. Close-up of the above showing penis sheath, supporting framework, and bases of hamules. ( $1 \times 0.10 \times E$ ).  
 Fig. 3. Photomicrograph of penis (lateral view) ( $10 \times 0.6 \times E$ ).  
 Fig. 4. Photomicrograph (close-up) of the penis tip ( $10 \times 0.10 \times E$ ).  
 Fig. 5. Photomicrograph of a portion of testis ( $1 \times 0.10 \times E$ ).  
 Fig. 6. Photomicrograph of 8th sternum showing gonopore, gonopods and genital plates, etc. ( $1 \times 0.6 \times E$ ).

**The Hamules.**—The hamules are a pair of prominent, stout appendages lying laterally in the genital fossa inbetween the anterior lamina and the genital lobes. Each hamule is a thick, laterally compressed and elongated organ with a laterally convex, strongly chitinized proximo-basal end (I) (Plate III, fig. 2). The basal region of each organ, however, is membranous on the mesal face. This allows, to some extent, a free movement of the hamule towards its fellow of the opposite side. Anteriorly each hamule is attached to a short ventro-lateral rod of the lateral arm of U-shaped framework and posteriorly it is articulated with the postero-basal ends of the penis sheath. Each of the hamules is bilobed distally; one is the anterior spur-like portion bearing at the tip a small strongly chitinized black hook which is curled inwards, and the other is a posterior, truncated, triangular portion with a rounded apex projecting caudad. The face of the truncated portion lies opposite the concave side of the spur-like portion. The anteriorly lying hook bearing portion is shorter in length than the posterior truncate portion. Large bristle-like hairs are found uniformly distributed on the upper surface of the hamule. The hamules maintain proper positions during mating.

**The Penis Vesicle.**—At the anterior end of the third abdominal sternite is a large somewhat hemispherical flask-like body, the vesicle of the penis (Vesicula spermalis of Tuxen, 1956) (Plate II, fig. 5) and (Plate III, fig. 1), measuring 1.071 mms. in length. It has a round bottom, which is divided into two lobes by a median cleft (Plate I, fig. 5). The dorsal plane surface of the vesicle is membranous and is attached for a greater length to the underlying wall of the abdomen. In the postero-median region there is a triangular area (N) (Plate I, fig. 4) on the dorsal side which shows indication of chitinization. The ventral surface of the penis vesicle is strongly chitinized and is divisible into two regions, one anterior triangular neck region (c) and the other posterior rectangular bulging region (d). Sclerotization in areas on the ventral surface of the penis vesicle is wanting in this case. However, on the ventral surface of the posterior one-third part of the posterior region of penis vesicle, two ventro-lateral areas of weak sclerotization are evident. Immediately anterior to these is a still weaker area of chitinization, running transversely through the penis vesicle and separating the lobed bottom from the body of the vesicle. The sub-ventral side of the posterior region of the penis vesicle bears large tough hairs (h). The vesicle does not open anteriorly into the genital fossa nor does it communicate with the body-cavity of abdomen. Its only communication is with the cavity of the penis. Penis vesicle stores sperms for the transference into the bursa copulatrix of the female.

**The Penis.**—Arising sub-terminally from the dorsal anterior end of the penis vesicle is a long segmented rod-like structure, the penis or prophallus (Tuxen, 1956) (Plate II, fig. 5) lying anteriorly in the groove of the penis sheath in the second abdominal segment. The penis is three segmented, the distal or the third joint lies bent over the first or the proximal segment (Plate III, fig. 3).

The first segment is a long stout rod measuring 0.663 mm. in length. This segment is dorsally placed and lies immediately anterior to the penis vesicle fitting closely in the groove of the penis sheath. It is slightly curved dorsally and bears sub-apically a prominent, strongly chitinized spur-like process, the dorsal process (W) on the meso-dorsal distal end. The dorsal and the lateral sides of the segment are strongly sclerotized, while the ventral surface is membranous.

The second segment is smallest of the three joints measuring 0.289 mm. in length. It lies antero-ventrally perpendicular to the distal end of the first segment. It is triangular in shape with a narrow membranous ventral surface and a rounded

dorsal border. Dorso-mesially it bears a weakly sclerotized depressed area. The rest of the dorsal and whole of the lateral surface is strongly chitinated.

The third or the distal segment is longest and most complicated of the three segments. This part of the penis is bent over the proximal one and measures 0.782 mm. in length. In a lateral view, this segment is narrow proximally and broad distally. This segment is divisible into two regions, an anterior conical region and a posterior rectangularly distended region. The anterior region is composed of two large sclerites which form a mid-ventral ridge towards the distal end. The posterior region bears dorsally a triangular convex sclerite. Laterally two horse-shoe shaped chitinous lobes are prominently present at the distal end of the penis. Dorsal to these is a pair of small chitinous sclerites bearing apically two long, very thin thread-like structures termed flagellum (Fraser, 1940) or cornua (Kennedy, 1922). These, however, disappear on treatment with KOH solution. The opening of the penis is simple and is borne at the end of a tube which protrudes well out of the terminal cluster of sclerites and lobes, (Plate III, fig. 4.)

## Discussion

### Testes :

As described above, each testis apparently appears unifollicular in nature, the follicle being represented by the single long cylindrical organ attached terminally to the vas deferens. But a histological examination of the organ reveals that it consists of numerous lobules (or cysts of Marshall, 1914) packed together so compactly that they have become indistinct externally. Moreover, each lobule contains a large number of germ cells and is connected to a common central duct by a small efferent ductule. According to Imms (1957), the lobules and follicles are synonyms. Evidently, the true nature of the testis in *L. asiatica asiatica* Fabr. is multifollicular instead of the apparent unifollicular.

Marshall (1914) describes the testes in *Libellula quadrimaculata* Linn. arising in the posterior part of the third abdominal segment and running slightly dorsal to the mid-intestine upto the posterior margin of the seventh abdominal segment. In *Lathrecista asiatica asiatica* Fabricius, however, the testes extend ventro-laterally to the alimentary canal from the posterior half of the sixth abdominal segment upto the end of the seventh abdominal segment.

Prasad and Srivastava (1960) mentioned the presence of fat bodies and tracheae attached in irregular groups to the wall of the testis in *Pantala flavescens* Fabr. In the species under investigation, the adipose tissue alongwith the tracheal ramification forms a thick and complete covering round the testis wall, helping probably in the nourishment of the developing germ cells inside.

George (1928) reported the central duct running upto the middle region of the testis in *Agrius*. In the insect under study, however, the central duct runs straight throughout the length of the testis.

### Vasa Deferentia :

Tillyard (1917) reported in *Aeschna* that before joining the sperm-sac, each vas deferens formed loops. In *L. asiatica asiatica* Fabr., the vasa deferentia do not form any loop before joining the sperm-sac. This is probably due to the fact that in *Aeschna* the sperm-sac is comparatively less developed and the spermatozoa are stored in the vas deferens also, which is, therefore, lengthened by the formation of loops. On the other hand no such necessity arises in *L. asiatica asiatica* Fabr. in which the sperm-sac is large and well developed.

### *Sperm-sac :*

The names Seminal vesicle (Marshall, 1914), Sperm-sac (Tillyard, 1917) and Vesicular-sac (George, 1928) are applied to the structure formed by the union of the two vasa deferentia in the middle line of the ninth abdominal segment. As has been observed, the point of union is clearly indicated externally by a notch and internally by a ridge bearing large columnar cells which are glandular also. The secretory function of the accessory glands which are absent in *L. asiatica asiatica* Fabr. is performed by these cells.

Marshall (1914) and Tillyard (1917) report that the sperms are present in bundles forming spermatophores inside the sperm-sac. In the present species, however, no spermatophores have been found in the sperm-sac. On the other hand, the sperms are present scattered irregularly in the lumen of the sac.

### *The External Genitalia :*

The minute shallow cup-like, somewhat triangular structure lodging the ejaculatory duct in its cavity and described earlier has been overlooked by all the previous workers. Snodgrass (1935) seems to refer this structure when he mentions the occurrence of a rudimentary penis in Odonata bearing the genital opening at the tip. George (1928) also reports the appearance of "a pair of tubercles situated between the rudiments of the coxites on the ninth sternum" in the early larval stages of *Agrion*. In the modern dragonflies, the copulatory function has been transferred from the ninth abdominal segment to the second abdominal segment, rendering thereby the true (primary) penis unfunctional, which finally degenerated. In view of these facts, the author regards the cup-like organ under discussion as homologous with the vestige of the original true penis. This organ is ineversible and very different from the typical penile form, but it is a known fact that structures may assume peculiar and very different forms as a result of degeneration. The exact nature of this structure can only be revealed by working out its development. In the present paper, however, the term penis has been used for the intro-mittent organ of the secondary copulatory apparatus, following the terminology of a majority of workers of Odonatology viz. Thompson (1908), Tillyard (1917) and Chao (1953) etc. The orifice of the penis in *L. asiatica asiatica* Fabr. is situated at the end of a tube which protrudes well out of the terminal cluster of sclerites and lobes, and not on the convex dorsal side of the second segment as described by Tillyard (1917) in *Aeschna*.

The pear-shaped appendages surrounding the male gonopore have been termed valvules by Tillyard (1917), coxites by George (1928) and gonapophyses by Tuxen (1956). However, they all regard them as vestigial gonapophyses of male dragonflies. Snodgrass (1957) has shown clearly that the gonapophyses develop separately and have no relation with the abdominal legs. The term coxite meaning "coxal part of a leg" is, therefore, misleading and should be discarded. George (1928) describes the presence of styli at the tip of these structure in early nymphal stages of *Argion*, which, however, disappear in the adult stage. Considering this and the fact that a rudimentary true penis\* exists in the dragonfly, these appendages represent the reduced gonopods, a view also shared by Snodgrass (1935).

The secondary copulatory apparatus differs from that of other species in the structure and shape of the anterior lamina, the hamules and the penis sheath, which can be regarded as specific for *Lathrecista asiatica asiatica* Fabr.

---

\*According to Snodgrass (1935), phallus is formed by the fusion of the gonapophyses.

The penis sheath has been termed Ligula by Tuxen (1956), as he regards that it functions as a director for the penis during intromission. Earlier workers like Tillyard (1917) and George (1928) have ascribed it a protective function because it lodges the penis in its groove. It is very likely that this organ functions both as a protective sheath for the penis when not in use, and as a director for it during intromission. For this reason, the author has preferred the term penis sheath over ligula of Tuxen (1956) for the structure under consideration.

As described earlier, each supra-anal appendage shows distally a row of 4-6 black small teeth-like protruberances on the ventro-lateral margin. These protruberances which have been overlooked by all the previous workers, probably provide a rougher surface to the organs used as claspers (Tillyard, 1917) during mating. Since they have been observed occurring constantly only in males, they seem to represent secondary sexual characters.

### Summary

The testes are cylindrical multifollicular organs, lying ventro-laterally to the alimentary canal in the posterior half of the sixth and the whole of the seventh abdominal segments. The vasa deferentia are simple tubes, each continued anteriorly inside the testis as central duct and opening posteriorly into a well developed vesicula seminalis. The two vesiculae seminales are united in the middle of the ninth abdominal segment to form a sperm sac. The ejaculatory duct is very small and cup-shaped situated in the centre of the ninth abdominal segment and connects the sperm-sac ventrally to the exterior. The accessory glands are totally absent. Male genital opening is an elongated spindle-shaped orifice situated inbetween a pair of highly sclerotized pear-shaped gonopods in the middle of the ninth sternum. A reduced 'primary penis' in the form of a deep chitinous cup is present dorsal to the male gonopore. The supra-anal appendages are well developed and bear ventro-laterally 4-6 black small teeth-like protruberances of a secondary sexual nature. The shape and the structure of the secondary copulatory apparatus is characteristic of the species studied. The functional intromittent organ ('secondary penis') is distinctly three segmented, the third segment being bent over the first. Its orifice is borne at the end of a tube which protrudes well out of the terminal cluster of sclerites and lobes.

### Aknowledgments

The author is greatly indebted to Dr. S. N. Prasad, Department of Zoology, University of Allahabad for his guidance and keen interest in the present work. He is also thankful to Prof. M. D. L. Srivastava, Head of the Zoology Department, Allahabad University for providing the necessary facilities and to Prof. D. S. Srivastava, Head of the Zoology Department, Saugar University for his valuable suggestions.

### References

- Chao, H. F. The external morphology of the dragonfly, *Onychogomphus ardens* Needham. *Smiths. Miscell. Coll.*, **122** : No. 6, 1-56, 1953,  
Fraser, F. C. The Fauna of British India, including Ceylon and Burma. Taylor and Francis Ltd., Red Lion Court, Fleet Street, London, E. C. 4, Odonata, **III** : 1936.  
——— A comparative study of the penes of the family Gomphidae (Odonata). *Trans. R. ent. Soc. London*, **90** : 541-550, 1 fig., 6 pls. 1940.

- George, C. J. The morphology and development of the genitalia and genital ducts of Homoptera and Zygoptera as shown in the life-histories of *Philaenus* and *Agrion*. *Quart. J. micros. Sci. London*, **72** : 447-485, 3 pls., 1928.
- Goddard, M. F. On the second abdominal segment in a few Libellulidae. *Proc. Amer. philos. Soc.*, **35** : 205-212, pls. XIV and XV, 1896.
- Grasse, PP. Traite de Zoologie, Anatomie, Systematique, Biologie. 9. Odonata by L. Chopard, Masson et cie Paris, 311-54, 1949.
- Imms, A. D. A General Textbook of Entomology. Methuen and Co. Ltd., 36, Essex Street, Strand, London, W. C. 2, Ninth Edition, pp. 886, 1957.
- Kennedy, C. H. The morphology of the penis in the genus *Libellula* (Odonata). *Ent. News*, Philadelphia, **28** : 9-14, pls. (ii) and (iii), 1922.
- Machotin, A. Zur morphologischen Bedeutung der ausseren Geschlechtsanhänge von Odonata and *Carausius morosus*. *Rev. Zool. russe. Moscow*, **9** : 23-84, 36 figs. 1929.
- Marshall, Wm. S. On the anatomy of dragonfly, *Libellula quadrimaculata* Linn. *Madison Trans. Wis. Acad. Sci.*, **17** : Part II, 755-790, pls. 68-81, 1914.
- Palmen, J. A. Über paarige Ausführgänge der Geschlechtorgane bei Insekten, *Helsingfors*, 1884.
- Prasad, S. N. and Srivastava, B. K. The morphology of male reproductive organs of *Pantala flavescens* Fabr. (Libellulidae : Odonata). *Proc. Nat. Acad. Sci. India*, Sec. B, **30** : Pt. III, 203-216, 1960.
- Selys-Longchamps, E. de and Hagen, H. A. Monographie des Gomphines. *Brussels*, 1-460, 23 pls., 1858.
- Snodgrass, R. E. Principles of Insect Morphology. McGraw-Hill Book Co., Inc. New York and London, 1-667, 319 figs., 1935.
- A revised interpretation of the external reproductive organs of male insects. *Smiths. Miscell. Coll.*, **135** : No. 6, 1-60, 1957.
- Srivastava, B. K. Studies on the reproductive system of certain Odonata. *D.Phil. Thesis*. (unpublished) Allahabad University, India, 1960.
- Thompson, O. S. Appendages of the second abdominal segment of male dragonflies (Odonata). *Rep. Ent. No. 23 in Albany, N. Y. St. Educa. Dept. Mus. Bull.*, No. 24, 249-263, 1908.
- Tillyard, R. J. The Biology of dragonflies. *Combridge Memoir*, xii+396, 188 text figs. 4 pls. 1917.
- Tuxen, S. L. Taxonomist's Glossary of Genitalia in Insects. Ejnar Munksgaard, Copenhagen, 1956.

A NEW INDIAN SPECIES OF *PERIPSOCUS* HAGEN, 1866  
(PSOCOPTERA : PERIPSOCIDAE)

By

B. DATTA

*Zoological Survey of India, Calcutta*

[Received on February 25, 1963]

**Introduction :**

The entomologists in this country have not devoted enough attention to the study of psocid fauna, partly because of minor economic importance. The contributions to our knowledge of Indian psocids are due to Enderlein (1903), Needham (1909), Banks (1914), and Menon (1941). As regards the Oriental fauna of the family Peripsocidae, Enderlein (1903) described *Peripsocus similis* End. *P. Reicherti* End. from Singapore. He also listed *P. piger* Hag. and *P. aethiops* Hag., originally described by Hagen from Ceylon, although placement of these two species either in *Peripsocus* Hagen (1866) or in *Ectopsocus* MacLachlan (1899) are contradictory in view of inadequate original descriptions. Present author made a taxonomic study of psocids collected on tea crops from N. E. India.

*Peripsocus sinensis* sp. nov.

**Female :**

*Colouration (freshly killed, in alcohol)* : Head pale cream, with light brown distinct semi-circular marking on inner margin of each eye and midway on each side of epicranial suture (Text-fig. 1, a). Vertex posteriorly bordered with light brown colouration. Epicranial suture dark brown. Postclypeus apparently with four diagonal light brown streaks on each side. Anteclypeus white. Labrum slightly darker at middle. Genae pale cream. Antennae brown, scape and pedicel comparatively darker. Maxillary palpi brown. Eyes black, inner edges of each lateral ocelli darkly pigmented. Meso and metathorax with dark brown tergites, scutella with lighter pigmentation. Coxae light brown, trochanters colourless, femora yellowish-white, tibiae and tarsi brown.

*Forewing* (Text fig. 1, b) : Smoky, pterostigma slightly darker, basally transparent. Stigmasac dark brown. An ill-defined transparent band extending from proximal part of pterostigma to Cu.

*Hindwing* (Text fig. 1, b<sub>1</sub>) : Paler, proximal  $\frac{1}{3}$  of costal cell and part over 'r' darker.

*Abdomen* : Ground colour pale cream, with 4-5 broken transverse brown bands,  $\frac{2}{3}$  part of epiproct transparent, rest brown.

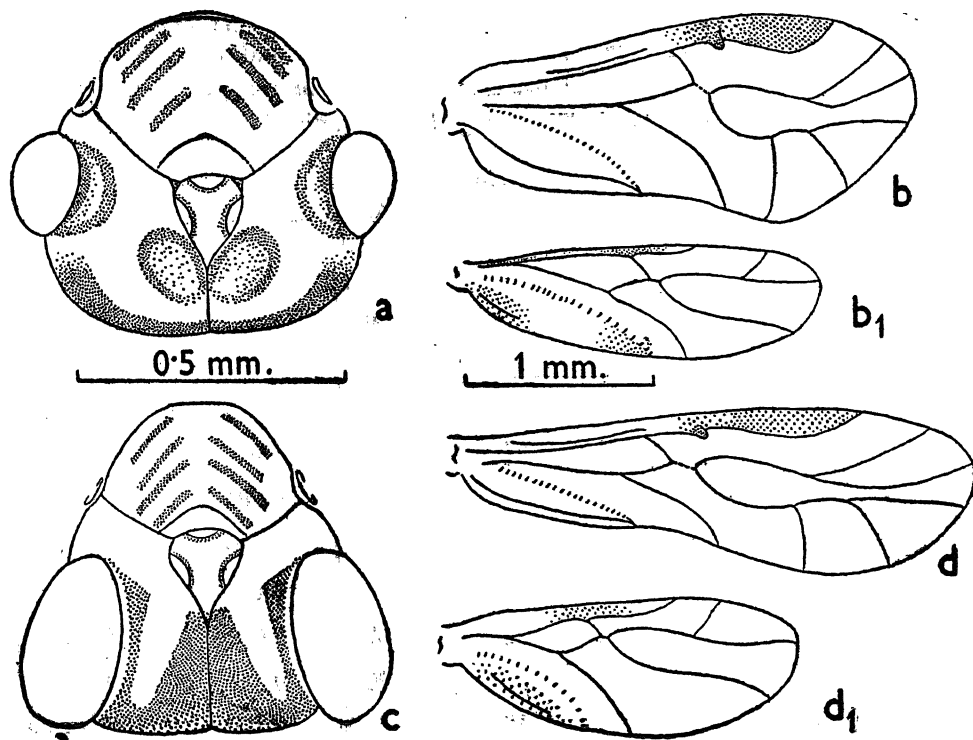
*Morphology* : Epicranial suture fine, distinct. Clypeus slightly protruding. Postclypeal area almost circular. Apical segment of maxillary palpi club-shaped, third segment shorter than other segments, all hairy.

*Forewing* : With microchaetae, stigmasac small, prominent. Along margin of 'an' a distinct transparent line.

*Hindwing* : With microchaetae.

*Ctenidiobothria* : Average 12 (9 examined).

**Subgenital plate** (Text fig. 2, *a*): Apically bilobed, each lobe dorsally bearing 13-14 setae, invaginated in form of 'U'. Behind apical lobe presence of a semi circular transparent area having short spines extending to apex of each lobe. Each lobe well-sclerotised. Long setae arranged sinuately along anterior border. Outer edge of each arm thinly sclerotised.



Text Fig. 1

**Conapophysis** (Text fig. 2, *b*): first valvula styliform, well-sclerotised, ventrally with numerous short spines of unequal sizes. Second valvula cordate-oval, dorsally with 10-11 long setae (avg. 11, five examined) ventrally with moderately stout spines. Third valvula small, cup-shaped, with 7-9 long setae (avg. 8, five examined).

**Epiproct** (Text fig. 2, *c*): Posterior half heavily sclerotised, distinctly marked from the anterior half. Surface with minute short spines. Outer edge semi-circular.

**Paraproct**: Trichobothria 21 average (three examined).

#### Male:

**Colouration** (freshly killed, in alcohol): Head pale brown, light brown marking along each side of epicranial suture and inner margin of each eye confluent anteriorly leaving a faintly yellowish streak in between two (Text fig. 1, *c*). Other parts concolourous with females.

**Forewing** (Text fig. 1, *d*);

**Hindwing** (Text fig. 1*d*<sub>1</sub>).



*Morphology* : Head narrower anteriorly, eyes larger than females.

*Ctenidiobothria* : 15 average (five examined).

*Genitalia* (Text fig. 2, d) : Penial frame closed anteriorly, external parameres fused posteriorly forming a beak, a pair of long distinct unsclerotised internal arms closely approximated together in the middle. Penial complex composed of three pairs of sharply pointed bifid hooks, each posterior pair with a pectinate lateral appendage. First and second pair equal, third pair longer.

*Epiproct* (Text fig. 2, e) : Unsclerotised, with minute spines and setae.

*Paraproct* : Trichobothria 33 average (two examined).

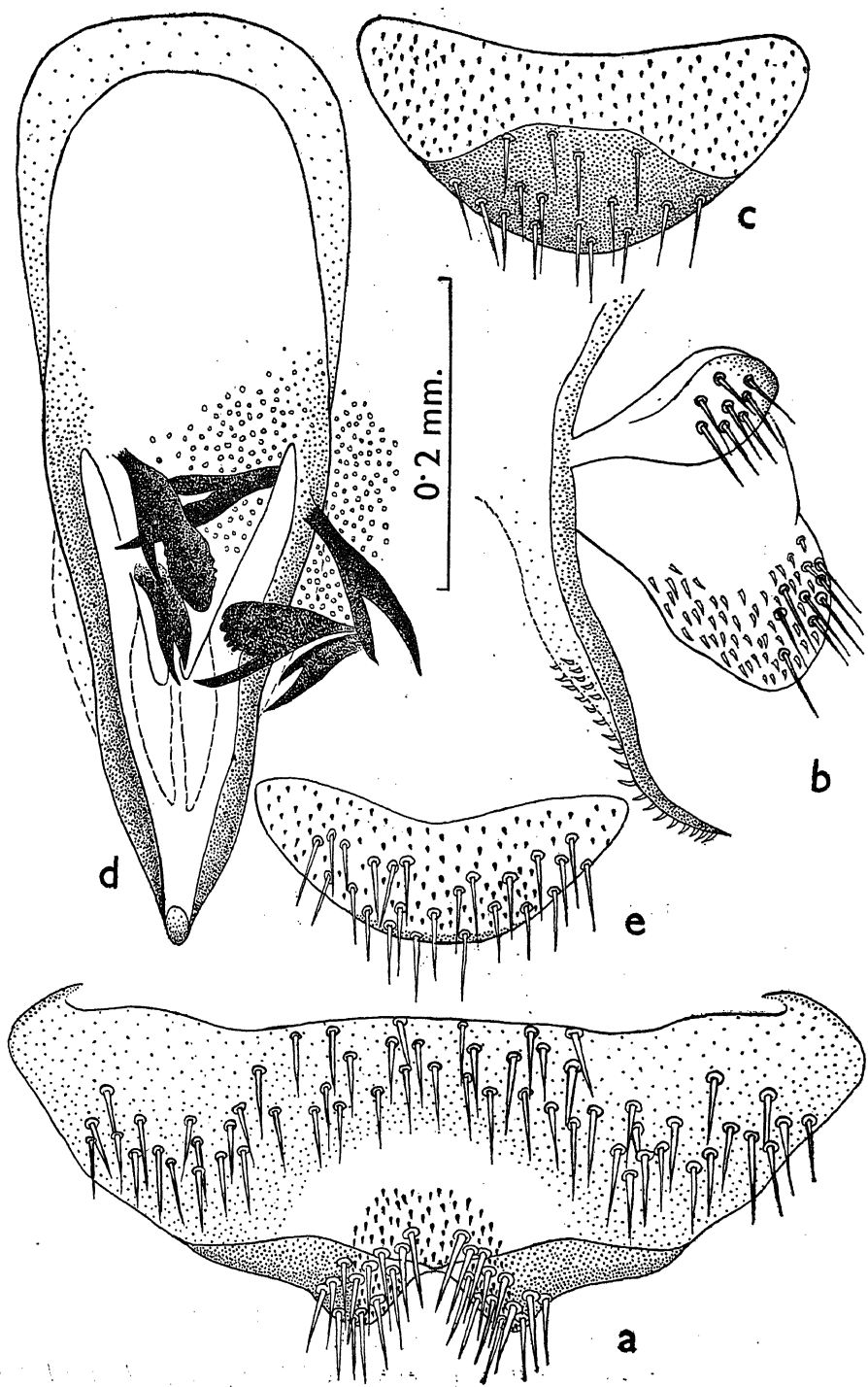
TABLE I  
Linear measurement of *Peripsocus sinensis*  
FEMALE

Character	N	Mean (mm.)	Standard deviations	Extremes
Head width	24	0.574	0.024	0.545-0.623
Interocular distance (10)	24	0.346	0.016	0.308-0.378
Eye-diameter (D)	24	0.113	0.008	0.098-0.133
Ratio 10/D	22	3.02	0.230	2.53 -3.33
Antennal length	12	1.20	0.086	1.16 -1.33
$f_1$	12	0.237	0.011	0.224-0.252
$f_2$	12	0.166	0.015	0.140-0.196
Ratio $f_1/f_2$	12	1.44	0.100	1.29 -1.60
Forewing	21	2.11	0.120	1.89 -2.41
Hindwing	11	1.73	0.097	1.58 -1.89
Hind femur	8	0.383	0.037	0.336-0.420
Hind tibia	8	0.759	0.057	0.672-0.840
Hind tarsus ( $t_1$ )	8	0.173	0.015	0.147-0.189
Hind tarsus ( $t_2$ )	8	0.087	0.007	0.084-0.105
Ratio $t/t_2$	8	2.01	0.180	1.75 -2.25

Body length (in alcohol) : 2.00 mm. (five measured).

#### Remarks :

Genitalia in males and females must be considered reliable as diagnostic features for specific determination. The other point of interest is the ratio of interocular distance to apparent eye-diameter for separating males and females belonging to same species.



Text Fig. 2

**TABLE II**  
**Linear measurements of *Peripsoeus sinensis***  
**MALE**

Character	N	Mean (mm.)	Standard deviations	Extremes
Head-width	12	0.570	0.023	0.532-0.616
Interocular distance	12	0.213	0.019	0.168-0.245
Eye-diameter	12	0.179	0.018	0.161-0.224
Ratio 10/D	12	1.13	0.210	0.73 -1.42
Antennal length	8	1.52	0.168	1.30 -1.76
$f_1$	9	0.277	0.031	0.238-0.336
$f_2$	9	0.207	0.044	0.182-0.231
Ratio $f_1/f_2$	9	1.34	0.070	1.20 -1.45
Forewing	12	2.11	0.107	1.89 -2.22
Hindwing	7	1.68	0.107	1.58 -1.89
Hind femur	8	0.373	0.077	0.336-0.420
Hind tibia	7	0.774	0.070	0.672-0.861
Hind tarsus <sub>1</sub>	8	0.202	0.011	0.189-0.210
Hind tarsus <sub>2</sub>	8	0.097	0.011	0.084-0.105
Ratio $t_1/t_2$	8	2.10	0.220	1.80 -2.50

Body length (in alcohol) : 1.78 mm. (two measured).

**TABLE III**  
**Relative position of the fork of radial sector with reference to the origin of  $M_2$  (Expressed in percentages)**

Species	Proximally	Opposite	Distally	Specimen examined
<i>P. sinensis</i> (female)	38.46	61.54	0	26
<i>P. sinensis</i> (male)	46.2	53.8	0	13

Dooars, N. Bengal India : On *Camellia sinensis* (L), vi. 60.

Holotype ♀, allotype ♂ and paratypes (♂♂, ♀♀) in due course will be deposited in the Zoological Survey of India.

#### Acknowledgments :

The work has been carried out in the Department of Entomology, Zoological Survey of India, Calcutta, I wish to express my thanks to Dr. M. L. Roonwall, Director, for the laboratory facilities he has afforded me. I am also indebted to Dr. M. S. Mani for his kind suggestions on the taxonomic study of psocids.

#### References :

- Badoneli, A. Psocoptères. Faune Fr., 42 : 1-164, 1943.  
 Broadhead, E. and Datta, B. The taxonomy, and Ecology of British species of *Peripsoeus* Hag. (Corrodentia) *Trans. Soc. Brit. Ent.*, 14 (5) : 131-146, 1960.  
 Enderlein, G. Die Copeognathen des Indo-Australischen Faunengebietes. *Ann. hist-nat. Mus. Hung.*, 1 : 179-344, 1903.  
 Menon, R. Studies on Indian Copeognathen (Psocoptera). *Indian J. Ent.*, 3 (1) : 13-23, 1941.

# FISHES OF CHOTANAGPUR (PART I), SADAR SUB-DIVISION RANCHI, BIHAR

By

C. M. SRIVASTAVA and V. R. P. SINHA

*Department of Zoology, Ranchi College, Ranchi*

[Received on February 14, 1962]

The division of Chotanagpur lies between  $83^{\circ}20'$ – $87^{\circ}45'$  longitudes and  $25^{\circ}15'$ – $20^{\circ}20'$  latitudes. It is bound on the north by Patna and Bhagalpur divisions, on the south by Orissa, on the west by Madhya Pradesh and on the east by West Bengal.

Chotanagur is often referred to as a plateau but this plateau extends beyond the limits of the division. It comprises several plateau surfaces. There are actually four plateaus in the stricter acceptance of the term—two in Ranchi and two in Hazaribagh elsewhere the country is often broken and numerous ranges or groups of steephills are intersected by deep ravines or occasionally by open valleys. The Geological formation is gneiss freely interbedded with micaceous, siliceous and hornblendic schists passing into metamorphic rocks in west Bengal and south Bihar.

## River systems :

The district of Ranchi occupies the southwestern corner of the province of Bihar. It extends from  $23^{\circ}0'N$  to  $23^{\circ}43'N$  and from  $84^{\circ}23'15''E$  to  $85^{\circ}54'E$ . It is more than 90 miles from east to west and 38 miles from North to South. The river systems of Ranchi plateau have their sources on the upward of Nagri-Ratu area, which is above 2,350' and all the rivers radiate in the East, West, North and South directions from it.

Among the eastward flowing rivers the Subernrekha is prominent. It has its source in a spring about a mile and half south of Nagri. It flows in this North-east direction and passes through the town of Ranchi. In its meandering course, receives the Raru river on its right bank, at a point, which marks the sub-divisional boundary in the south-east. The Subernrekha has numerous affluents, and has a large catchment basin in the dense reserved forest of Horhap. It receives the Ganganadi from the left side, below the Johna falls. Another important stream—Uraongarha Nadi flows into the Subernrekha near Muri, from the west.

The South Koel is the main Westward flowing river. It starts from the vicinity of Daladali Tea Gardens, about  $4\frac{1}{2}$  miles south of Ratu. It is fed on both sides by the Bandora Nadi, the Sapahi, the Tati, the Banki, and the Phuljhar.

In the northern *Thanas* of Bermu, Ormanghi, and the northern part of the Sadarthana, the streams have a general tendency to flow towards the north. The Damodar river flows beyond the district and at places form the boundary between the district of Ranchi and Hazaribagh. Among the numerous streams the Sapahi, the Batuka and the Nakri are important. The first two flow into the Damodar river while the last into the Nalkari Nadi, an affluent of the Damodar. The Damodar, the Mur and Garha Nadi are the main tributaries of the Sapahi. The Garha and the Sapahi are perennial streams.

The Karo is the main southward flowing river, with its origin in a spring near Katarpa, 2 miles south of Nagri. The Lohargara Nadi starts from Charhi Hill (2,313') at about 3 miles south of Bharno near the sub-divisional boundary. These two have numerous affluents from both sides. The stream courses are all sinuous and the general trend of flow is toward the south. The Lohargara receives the Bhangi Nadi at Baradhih  $1\frac{1}{2}$  miles west of Perwan Hill (2,349'). The Jamuni Nadi flows into the Karo  $1\frac{1}{2}$  miles east of Kakriya.

In the early course of the rivers near the edge of the plateau where they descend upon the lowlands, waterfalls have been noticed at several places. The principal waterfalls of the Ranchi Plateau are Hundru Ghagh, Johna, and Dumargarhi. Hundru is about 28 miles to the north east of Ranchi town. Here the Subernrekha falls down from a height of 243'. Johna is about a mile south of the Johna Railway Station. It is 85' in height. Dumargarhi lies below the Johna falls. Being situated in a dense forest, it is not easily accessible. It is on the Dumargarha Nadi. The water falls from a height of over 100'.

There is a total absence of natural lakes of any magnitude in the whole sub-division. The valleys and depressions have been dammed at places for the storage of water. The picturesque lake in Ranchi town has an area of 50 acres. There is an artificial lake north west of Kanke Agriculture farm. It is commonly called the serpentine lake. The rivers get swollen during the monsoons but for the greater part of the year have comparatively little water. Some of them completely dry up.

Tanks and ponds are numerous all over the Ranchi Plateau, particularly in the eastern part. Several new schemes of damming rivers and streams for irrigation purposes have been undertaken by the Government and some have been completed; e.g., the Kanke Dam across the Potpote, an affluent of the Jumar. This Kanke reservoir meets the water supply of the Ranchi town.

#### Systematic List :

The fishes were collected from the various parts of the district during the year 1960-62 in different seasons, mostly during October to February when the catch is the maximum. Altogether 33 species of fishes belonging to 12 families have been recorded. A classified list of the fishes collected is presented below. The authors have followed the classification of L. S. Berg.

	Scientific Name	Local Name
<i>Order</i> : Cypriniformes		
<i>Sub-order</i> : Cyprinoidei		
<i>Family</i> : Cyprinidae		
	<i>Labeo rohita</i> (Hamilton)	... Rohu
	<i>Labeo Calbasu</i> (Hamilton)	... Kalbose
	<i>Labeo bata</i> (Hamilton)	... Bata
	<i>Labeo gonius</i> (Hamilton)	... Karchia
	<i>Barilius bola</i> (Hamilton)	
	<i>Catla catla</i> (Hamilton)	... Catla
	<i>Cirrhina reba</i> (Hamilton)	... } Mrigal
	<i>Cirrhina mrigala</i> (Hamilton)	

	Scientific Name		Local Name
	<i>Oxygaster bacaila</i> (Hamilton)	...	Chalwa
	<i>Puntius sarana</i> (Hamilton)	}	Pothia
	<i>Puntius stigma</i> (Cuv and Val)		
	<i>Puntius ticto</i> (Hamilton)		
	<i>Danio rerio</i> (Hamilton)		
	<i>Danio telera</i> (Hamilton)		
	<i>Garra annandela</i> (Hamilton and Buch)		
Family : Cobitidae			
Sub-family : Nemachilini			
	<i>Nemacheilus botia</i> (Hamilton)	...	Tel-murrey
Sub-family : Cobitini			
	<i>Lepidocephalichthys guntea</i> (Hamilton)	...	Gete Buddha
Sub-order : Siluroidei			
Family : Siluridae			
	<i>Wallagonia attu</i> (Bloch and Schneider)	...	Boari
Family : Bagridae			
	<i>Mystus seenghala</i> (Sykes)	...	Tengra
	<i>Mystus aor</i> (Hamilton)	...	
	<i>Mystus vittatus</i> (Bloch)	...	
Family : Saccobranchidae			
	<i>Heteropneustes fossilis</i> (Bloch)	...	Seengi
Family : Clariidae			
	<i>Clarius batrachus</i> (Linn)	...	Maguri
Order : Ophicephaliformes			
Family : Ophicephalidae			
	<i>Ophicephalus gachua</i> (Hamilton)	...	Garai
	<i>Ophicephalus punctatus</i> (Bloch)	...	
	<i>Ophicephalus striatus</i> (Bloch)	...	
Order : Clupeiformes			
Sub-order : Notopteroidei			
Family : Notopteridae			
	<i>Notopterus chitala</i> (Hamilton)	...	Palat
	<i>Notopterus notopterus</i> (Pallas)	...	
Order : Perciformes			
Sub-order : Gobinoidei			
Family : Gobiidae			
	<i>Glossogobius giuris</i> (Hamilton)	...	Bulla
Order : Mastacembeliformes			
Family : Mastacembelidae			
	<i>Mastacembalus armatus</i> (Lacepede)	...	Gaichee
	<i>Mastacembalus pancalus</i> (Hamilton)	...	

	Scientific Name	Local Name
Order : Symbranchi-formes		
Sub-order : Symbranchoidae		
Family : Amphipnoidae		
	<i>Amphipnous cuchia</i> (Hamilton)	... Dugdugia
Order : Anabantoidei		
Family : Anabantidae		
	<i>Trichogaster fasciatus</i> (Bl. Schn)	

#### Summary :

Altogether 33 species of fishes have been recorded but only 13 of them are of economic value, judged from their size and weight. *Labeo rohita*, *Labeo calbasu*, *Labeo bata*, *Catla catla*, *Cirrhina mrigala*, are the most common representatives of the major carps. Important live fishes of food value are *Heteropneustes fossilis*, *Clarias batrachus*, *Ophicephalus punctatus* and *Ophicephalus gachua*. Among other varieties of fishes *Barbus* (Puntius) constitute, the major bulk.

Recently the fisheries department of the Government of Bihar has started the introduction of mirror carp in the region. The results have yet to be seen as the scheme is under experimental stage.

#### References :

- Berg, L. S. Classification of fishes both recent and fossil. *Trans. Inst. Zool. Acad. Sci. U. R. S. S. V.* : 87-517, 1940.
- Day, F. The fauna of British India-Fishes, 1889.
- David, A. On some records of fish from the Damodar and the Mahanadi river system. *J. Zool. Soc. India*, 5 : 243-254, 1953.
- Hora, S. L. On a collection of fish from the Rajmahal Hills Santhal Parganas, *Bihar Rec. Ind. Mus.*, 49 : 169-181, 1938.
- Imperial Gazetteer of India-Bengal Vol. II.
- Job, T. J., David, A. and Das, K. N. Fish and Fisheries of the Mahanadi in relation to the Hirakund Dam. *Ind. Jour. Fish.*, 2 : 1-36, 1955.
- Malaviya, R. B. A list of fishes from Jabalpur, M. P., India, *Proc. Nat. Acad. Sci. Ind.*, 31 : 349-354, 1961.
- Menon, A. G. K. On a collection of fish from the foot of Parahnath Hills, Chotanagpur. *Rec. Ind. Mus.*, XLVIII : 71-72, 1950.
- Pathak, Murari. Thesis-for the degree of Master in Arts in Geography Banaras Hindu University "Geographical investigation of Sadar sub-division, District Ranchi (Bihar)", 1960.
- Sinha, B. M. and Shirmony, P. A. The Fishes of Meerut. *Rec. Ind. Mus.*, 51 : 61-65, 1953.
- Srivastava, C. M. and Sinha, A. A. N. Fish and Fisheries of Ranchi Lake, Ranchi, Bihar. *Proc. Nat. Acad. Sci. Ind.*, 31 : 126-132, 1961.

EFFECT OF CERTAIN FUNGICIDES, ANTIBIOTICS AND SYNTHETIC  
PHYTOHORMONES ON THE GERMINATION OF CHLAMYDOSPORES  
OF *PROTOMYCES MACROSPORUS* UNGER CAUSING 'STEM-GALL'  
DISEASE OF CORIANDER

By

S. B. MATHUR and P. N. NARULA

Botany Department, Agra College, Agra

[Received on January 15, 1960]

**Introduction :**

During the last three decades preparations belonging to diverse groups of chemicals have been tested for their fungistatic properties by observing their influence on spore germination or mycelial growth. These preliminary laboratory trials are helpful in the evaluation of fungicides. Gupta (1958) noted complete inhibition of chlamydospore germination of *Protomyces macrosporus* Ung. at 1000 ppm. with Agrosan, Perenox, Streptomycin and Penicillin. In the present paper the evaluation has been extended to six fungicides (Blitox-50, Coppesan, Dithane Z-78, Wettle Sulphur, Aretan, Agallol); two antibiotics (Achromycin-Lederle; and Chloromycetin-Parke Davis), and three phytohormones (2,4-dichlorophenoxy acetic acid, Indole acetic acid and Naphthalene acetic acid). The parasite causes the 'stem-gall' disease of coriander (*Coriandrum sativum* L.) and the chlamydospores formed in the galls play an important role in the recurrence of the disease.

**Method and Material :**

Modified Rideal Walker's method (McCallan 1947) of evaluating antiseptics was followed. The chemicals were used in various concentrations ranging from 0 to 1000 ppm. To obtain a homogenous suspension of chlamydospores in water, heavily infected fruits showing characteristic hypertrophy were surface sterilized with 0.1% mercuric chloride solution for about a minute or so and repeatedly washed with sterilized distilled water in which the fruits were finally crushed. The extract was filtered through sterilized muslin cloth. The suspension drops, each containing 10 to 30 chlamydospores were placed on slides in three linear rows and allowed to dry up leaving behind the spores sticking to the slides. The slides were then transferred to Coplin jars containing the test solutions of different chemicals in various concentrations stored in a refrigerator at 19-20°C which is the optimum temperature range for germination of chlamydospores (Srivastava 1955). 25 observations for germination for each treatment were recorded on the tenth day. Standard errors for each concentration of a chemical have been calculated and included along with the 'means' in table I.

The data included in the above table clearly shows a progressive increase in percentage inhibition with increasing concentrations of the chemicals used. At 1000 ppm the inhibition in spore germination was 100% in all the chemicals except in Wettle Sulphur and Coppesan where it was 98.56% and 91.98% respectively. Even in low dilutions (10 and 100 ppm.), the effect of the various chemicals used was quite pronounced and inhibition was generally above 60%.



## Experimental

TABLE I  
Effect of different chemicals on percentage inhibition of  
germination of chlamydospores  
(Mean of 25 observations)

Chemicals	Concentration in ppm.		
	10	100	1000
Wettle Sulphur	62.22 $\pm$ 2.13	63.16 $\pm$ 2.45	98.56 $\pm$ 0.53
Blitox-50	90.88 $\pm$ 1.41	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00
Agallol	81.42 $\pm$ 2.13	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00
Aretan	97.35 $\pm$ 0.68	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00
Dithane	94.42 $\pm$ 1.56	98.30 $\pm$ 0.69	100.00 $\pm$ 0.00
Coppesan	72.94 $\pm$ 3.66	86.22 $\pm$ 1.32	91.98 $\pm$ 0.90
Achromycin	83.17 $\pm$ 1.22	91.62 $\pm$ 0.88	100.00 $\pm$ 0.00
Chloromycetin	78.66 $\pm$ 2.45	91.08 $\pm$ 1.51	100.00 $\pm$ 0.00
2,4-D	53.60 $\pm$ 1.25	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00
NAA	65.90 $\pm$ 1.38	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00
IAA	53.68 $\pm$ 1.23	85.36 $\pm$ 0.53	100.00 $\pm$ 0.00

Control (0 ppm.—Distilled water) for fungicides and antibiotics was 32.65  $\pm$  2.78

Control (0 ppm.—Distilled water) for phytohormones was 27.50  $\pm$  1.47

TABLE II  
Influence of various compounds on inhibition of chlamydospore-  
germination in *Protomyces macrosporus*  
(% over respective control)

Chemicals	10	100	1000
Achromycin	75.02	87.56	100.00
Chloromycetin	68.32	86.76	100.00
Wettle Sulphur	43.91	45.31	97.96
Blitox-50	86.46	100.00	100.00
Agallol	72.42	100.00	100.00
Aretan	96.07	100.00	100.00
Dithane Z-78	91.71	97.48	100.00
Coppesan	59.83	79.54	88.10
2,4-D	35.93	100.00	100.00
NAA	52.92	100.00	100.00
IAA	36.32	79.79	100.00

Table II reveals that all the compounds used except Wettle Sulphur, 2,4-D and IAA proved to be toxic at 10 ppm. (LD 50). The lethal dose of Wettle Sulphur, 2,4-D and IAA is 100 ppm.

#### Summary :

The influence of six fungicides (Blitox-50, Coppesan, Dithane Z-78, Wettle Sulphur, Aretan and Agallol), two antibiotics (Achromycin and Chloromycetin) and three synthetic phytohormones (2,4-D, NAA and IAA) in varying concentrations (0 to 1000 ppm.) upon the inhibition of germination of chlamydospores of *Protomyces macrosporus* Ung. has been investigated, using a modification of Rideal Walker's method of evaluating antiseptics.

All the compounds under study except Wettle Sulphur, 2,4-D and IAA showed inhibition of spore germination by 50% or more at 10 ppm. and thus they can be classified as effective fungistatic substances for the chlamydospores of *Protomyces macrosporus* Ung. in low dilutions.

#### Acknowledgements :

We are grateful to Dr. S. Sinha, Professor and Head of Botany Department. Agra College, Agra for his guidance and help during the progress of the work.

#### References :

- Gupta, J. S. Laboratory assay of certain fungicides, antibiotics and sulphadiazine on the germination of chlamydospores of *Protomyces macrosporus* Ung. *Agra Uni. Res. Jour. (Sci.)*, Vol. VII, Part I, 105-112, 1958.
- McCallen, S. E. A. Bioassay of certain fungicides. *Agri. Chemicals*, 2 (9) : 31, 67 ; 2 (10) : 45, 1947.
- Srivastava, H. C. Studies on Tumour Disease of *Coriandrum sativum* L. caused by *Protomyces macrosporus* Ung. *Proc. 42nd Ind. Sci. Cong.* Part III, p. 218. 1955.
- Trevelyan, J. W. The error of determination of toxicity. *Proc. Roy. Soc. Lond. Ser. B.*, 101 : 483-514, 1927.

SMUT DISEASE OF 'SAWAN', *ECHINOCHLOA FRUMENTACEA* LINK  
CAUSED BY *USTILAGO PARADOXA* SYD. AND BUTL.

By

B. B. SHARMA

Department of Botany, Lucknow University, Lucknow

[Received on May 3, 1962]

**Introduction :**

*Echinochloa frumentacea* Link (Syn. *Panicum frumentaceum* Roxb. ; *Panicum crus-galli* var. *frumentacea* (Roxb.) Trim.), locally known as 'Sawan' or 'Banti' is an important smaller millet crop extensively used by the poor as food and in U. P. alone covers 5,21,000 acres of land. It is sown in July and harvested in September—October. The 'Sawan' plants are infected by three species of *Ustilago*, viz. *U. paradoxa* Syd. and Butl., *U. panici-frumentacei* Bref. and *U. crus-galli* Tracy and Earle (Butler, 1918 and Mundkur, 1943). The three species of *Ustilago* are distinguished from each other by their region of infection on the host, spore size and character, their mode of germination and the nature of promycelium (Mundkur, 1943). Of these, *U. panici-frumentacei* is very wide in occurrence having been reported from Nyasaland, North and Central Asia and is also probably found in China, Japan, Russia, South Africa and North and Central America (Lavroff, 1936 ; Leach, 1932 and Ling, 1953). Of all the three species of *Ustilago*, *U. paradoxa* is the cause of most severe disease in 'Sawan', its sori being produced in the ovary. Butler (1918) gave a short account of *U. paradoxa* found in Pusa. It was later reported by Kulkarni (1922) from Bombay Presidency and Sind. The author, during the course of the survey of smut flora on cereals, has found *U. paradoxa* to be of common occurrence in Uttar Pradesh.

Kulkarni (1922) in his preliminary work on the life-history of *U. paradoxa*, reports the occurrence of seedling infection by the seed borne spores and recommends a treatment of the seeds with 2% solution of copper sulphate for 10 minutes as a successful measure of its control. In conformity with Kulkarni's observations, the author has also found the occurrence of the disease ranging from 35–40% when healthy spore free seeds of 'Sawan' were mixed with a concentrated suspension of *U. paradoxa* spores prior to sowing, and also when the smut spores were mixed in the soil (in pots) autoclaved three times at 20 lbs. pressure and externally sterilised seeds sown in it. *U. paradoxa* fails to secure infection through the hypocotyl, young leaf or the floral parts of 'Sawan', as has been evidenced by suitable infection experiments. It is thus apparent that it infects only through the radicle of the host seedling.

Study of the 'Sawan' smut caused by *U. paradoxa* was hence elaborated as presented here, with regard to the germination and viability of its spores, susceptibility of the host seedlings under seasonal temperature and as influenced by low temperature treatment prior to infection, histopathology and the varietal resistance of 'Sawan'.

# PLATE



Fig. 1.—'Sawan' plants in pots.  
 A. Infected plants with smutted floral spike presenting a compact appearance.  
 B. Healthy plants in which the lateral axes of the floral spike have a spreading tendency.

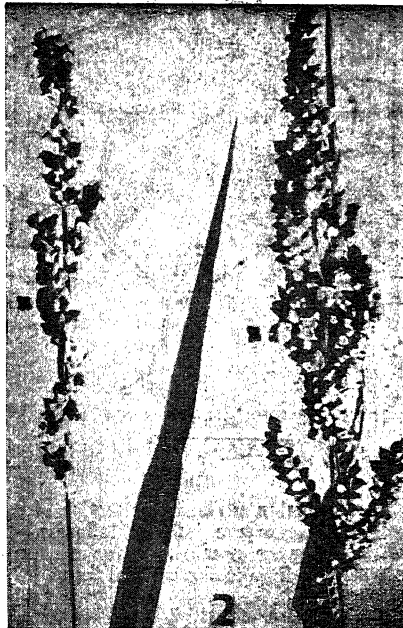


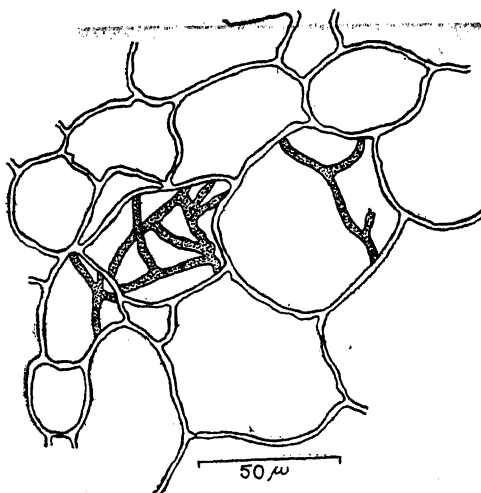
Fig. 2.—Smutted ears of 'Sawan' Showing the distinct hypertrophy of the affected grains (marked x).

### Material and Method :

For the experimental work smutted ears of 'Sawan', collected from local diseased fields, were stored in paper envelopes in desiccators. Spores were taken out by rupturing the smutted grains at the time of the experiment and treated externally first with a 10% silver nitrate solution for 5 minutes followed by 3% sodium chloride solution and sterilised distilled water for 5 minutes each. 'Sawan' seeds were obtained from their healthy fields and prior to sowing, were sterilised externally by the mercuric chloride treatment in the usual way. The percentage of disease was estimated on per individual plant basis even if only a few grains in the ear were smutted.

### Symptoms of Disease :

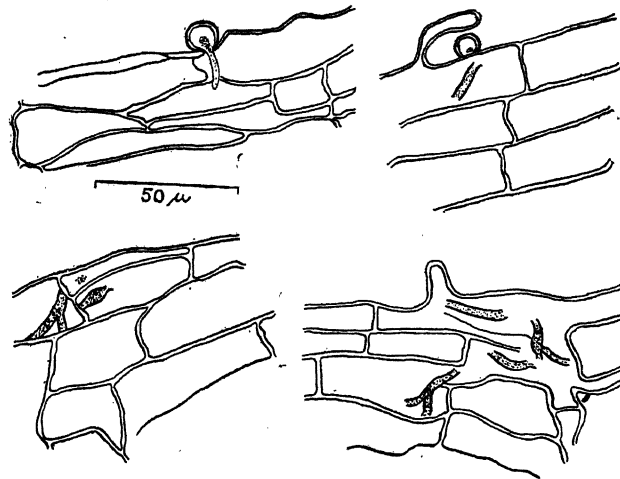
The disease is visible externally only at the maturing stage of grains in the ear. In general the infected floral spike presents a compact appearance as compared to healthy floral spike which has a tendency to spread out (Plate, Fig. 1). The rachis of the smutted ears is rather crooked and bent at places with longitudinal striations all over the surface. The smutted grains are slightly larger, sometimes 3-4 times, than the healthy ones (Plate, Fig. 2). The grains are replaced by powdery mass of spores loosely associated in lumps. The spores are nearly spherical or oval,  $7.8-11.2\ \mu$  in size with thin and smooth epispore. The outer surface of the covering glume of the smutted grains is rough, hairy and dirty grey.



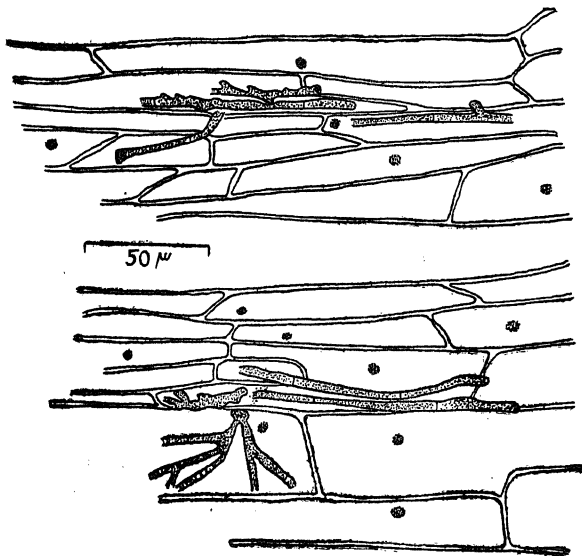
Text Fig. 1.—A portion of transverse section of the stem just below the infected inflorescence stalk showing the profusely branched intracellular mycelium in the cortical tissue.

The histopathological study of the diseased 'Sawan' plants revealed profuse occurrence of hyphae in the cortex and pith of the peduncle and the rachilla in the inter- and intra-cellular condition (Text Fig. 1). The course of the infecting hypha in the seedling stage was studied by Garrett's (1937) method of maceration; which showed the attachment of *U. paradoxa* spores all over the outer surface of the young

radicle, penetration of the hypha (germ tube) from the germinated brand spores through the epiblemma cells and their consequent spread in the inner tissues (Text fig. 2). The hyphae grow upto the meristematic shoot apex (in 8-10 days old seedlings) where they chiefly occur in inter- (sometimes intra-) cellular condition (Text fig. 3).



Text Fig. 2.—The macerated radicle of 2-3 day old seedling showing the germination, penetration and development of the parasite inside the tissue of the host radicle.

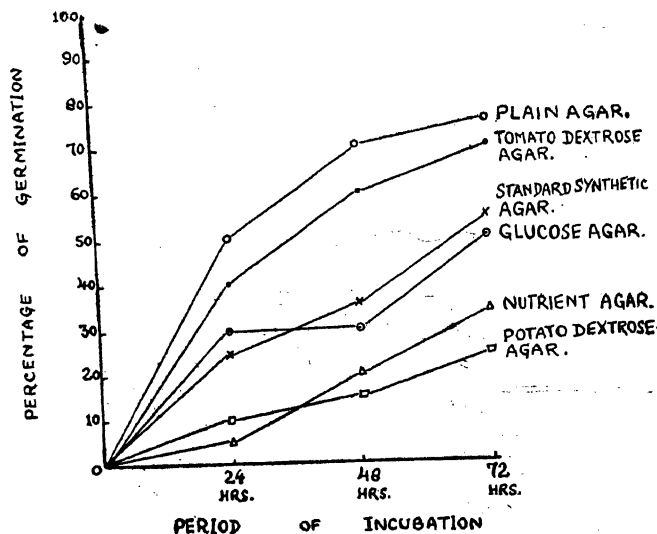


Text Fig. 3.—The nature of mycelium near the growing apex of the macerated radicle of 8 days old infected seedling.

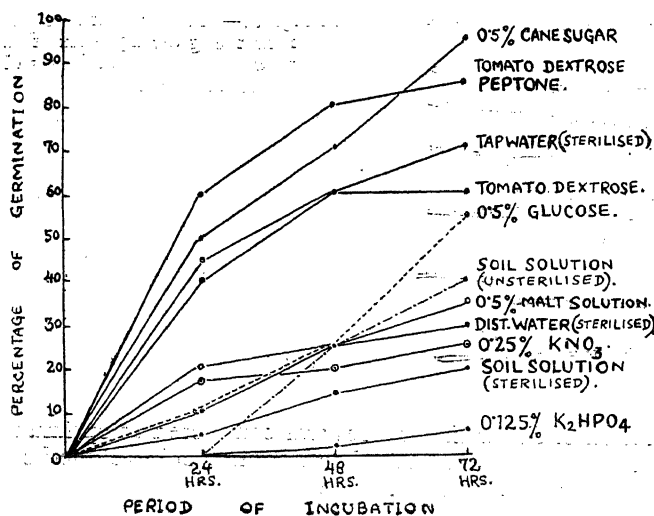
#### Germination of the Brand Spores :

The study of the germination of *U. paradoxa* spores was conducted at optimum temperature of 35°C by either the 'hanging drop' or the 'slide culture' method depending respectively whether the media used were liquid or solid.

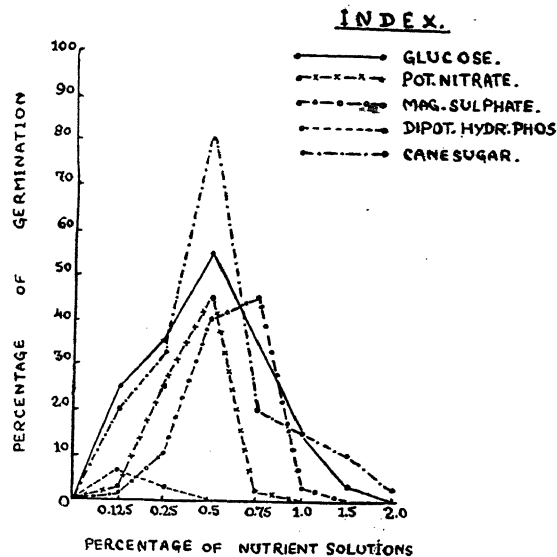
The brand spores, on germination, usually produce a short or elongate, unbranched or branched hypha (the promycelium), which remains aseptate or becomes septate. The production of sporidia has not been observed in any of the media utilised. In 0.5% potassium nitrate, sugar solution and the tap water the promycelium is branched while in the solid media it is long, almost straight or sometimes curved without much of branching (Text fig. 8.) The media best suited for germination are: plain agar, tomato dextrose agar, 0.5% sugar solution, 20% tomato + 2% dextrose +/- 1% peptone and 0.5% glucose and potassium nitrate solutions. In all concentrations used of dipotassium hydrogen phosphate the spores germinate with difficulty by producing a small bud like protuberance only as is also the case when they are subjected to 40° and 45°C temperature. The optimum temperature for germination has been found to be 33°-35°C and there is no germination at 50°C and 10°C (Text figs. 4, 5, 6 and 7).



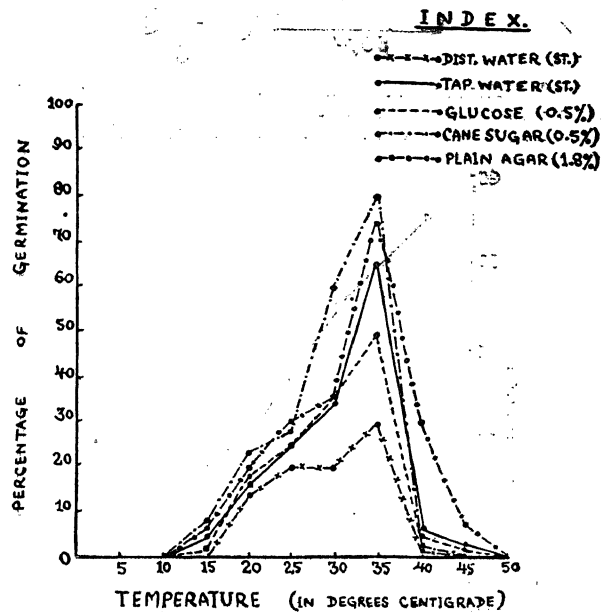
Text Fig. 4.—The effect of solid media on the germination of the brand spores of *U. paradoxa* at optimum temperature of 35°C.



Text Fig. 5.—The effect of the various liquid media on the germination of the brand spores of *U. paradoxa* at optimum temperature of 35°C.

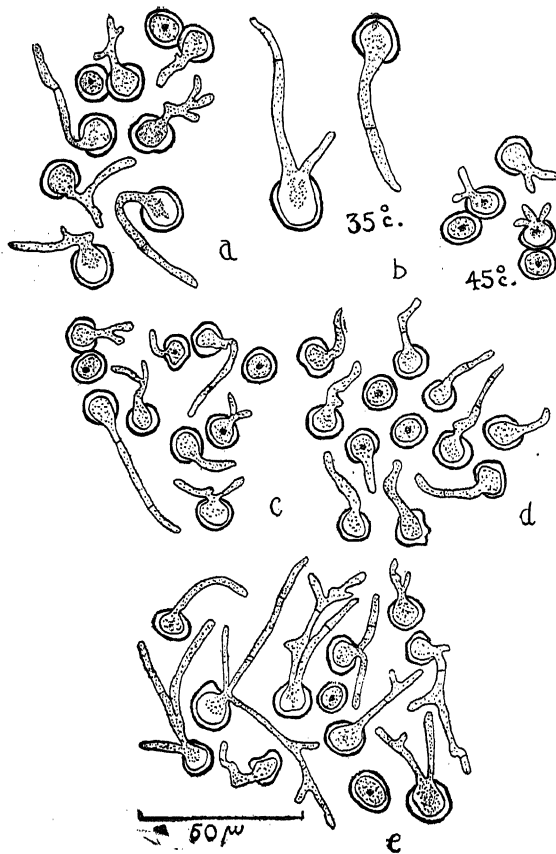


Text Fig. 6.—The effect of different concentrations of individual nutrients on the germination of the brand spores of *U. paradoxa* at optimum temperature of 35°C.

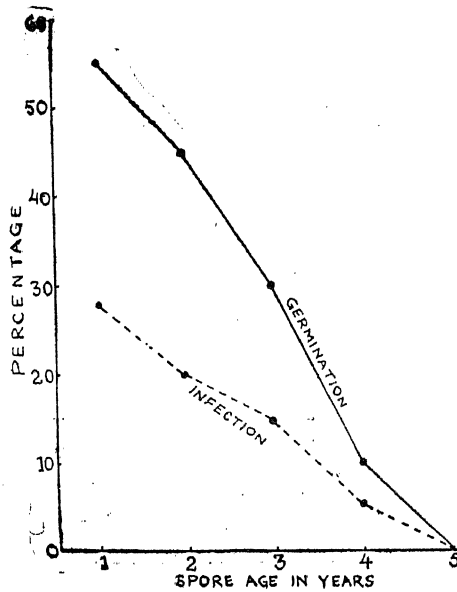


Text Fig. 7.—The effect of temperature on the germination of the brand spores of *U. paradoxa*.





Text Fig. 8. The mode of germination of the *U. paradoxa* spores at optimum temperature of 35°C in (a) tap water, (b) distilled water, (c) glucose agar, (d) plain agar and (e) 0.5% potassium nitrate solution.



Text Fig. 9. The effect of age on the viability of the spores of *U. paradoxa*.

### **Viability of Spores :**

For this purpose the spores of *U. paradoxa*, collected for 5 consecutive years from diseased fields, were preserved dry under laboratory conditions and their viability was determined by—

- (i) germination experiments in 'hanging drop' cultures using 0.5% glucose solution and incubating them at 35°C. (optimum temperature), and
- (ii) infection experiments, by sowing externally sterilised 'Sawan' seeds contaminated with spores.

The results presented in text figure 9 indicate that the brand spores remain viable for 4 years and that their viability falls down with age.

### **Susceptibility period of the 'Sawan' seedlings :**

Externally sterilised seeds of 'Sawan' were sown in autoclaved saw dust. The seeds sprout in 18–20 hours. Thirty seedlings were carefully removed from the saw dust every day till they were 18 days old. The radicle of the seedlings thus taken out in each case was immersed in spore suspension of *U. paradoxa* for 20 minutes and then the seedlings were transplanted in pots with sterilised soil.

It was observed that 2–3 days old seedlings produced the highest percentage (60%) of diseased plants while seedlings older than 6 days matured into disease free plants (Text fig. 10).

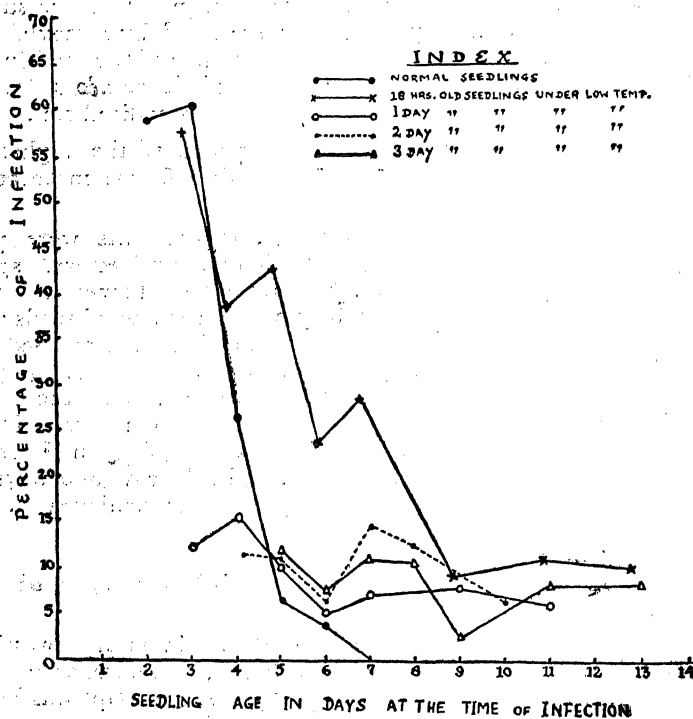
In order to give the parasite an advantage, experiments were performed in which smut spores of different ages of germination were mixed with sprouted seeds and sown. For this purpose spores of *U. paradoxa* were allowed to germinate in 0.5% glucose solution at 35°C. The healthy sprouted 'Sawan' seeds were sown in sterilised soil pots after they had been mixed with these germinated spores taken out after every 24 hours until 5 days. By this treatment the fungus is able to infect the seedlings immediately after it comes in contact with these in the soil. For each set in the above case 55 plants were allowed to mature. This treatment yielded a higher percentage of disease, the highest being 68% in the set in which 3 days old germinated spores were utilised and 45% in the case of 5 days old germinated spores; the former being the highest percentage achieved in all types of infection experiments.

### **Effect of low temperature on the Susceptibility period of the 'Sawan' seedlings :**

The experiments have thus shown that the infection of 'Sawan' seedlings by *U. paradoxa* decreases with age. The susceptibility, therefore, seems to be related to certain changes in the radicle of the seedlings. An attempt was hence made to arrest the growth of the seedlings by placing them in low temperature prior to infection. A large number of externally sterilised seeds were sown in saw dust. After sprouting (18 hours after sowing) these were removed inside a refrigerator set at 8–10°C. Light to the seedlings was supplied by lighting a 60 watt electric bulb inside the refrigerator for 8 hours period per day. Similarly, seedlings were allowed to grow in saw dust pans for 1 day, 2 days and 3 days respectively under normal conditions and then removed in the refrigerator.

From each of these four sets, 50 seedlings were taken out every day for 5 days and after that every alternate day till they had remained for 15 days inside the refrigerator. The radicle of the seedlings, from each of these sets, was dipped in the spore suspension of *U. paradoxa* and then they were planted in garden pots.

Thus at the time of sowing, the seeds are in the germinated condition (18 hours to 3 days old) but the spores, with which they are associated are still ungerminated and shall germinate 18-24 hours after reaching in the soil. Therefore the age of the seedlings, at the time of infection by the smut parasite, would have increased by one day more than what it was at the time of transplantation. The results have accordingly been shown in text figure 10; which show that the low temperature treatment of the 'Sawan' seedlings, prior to infection, on the one hand, depresses their susceptibility while on the other hand it increases the period of infection. Under normal conditions the infection in 2-3 days old seedlings is high-40%—but it is only 10% when they have been subjected to low temperature. Normally there is no infection of seedlings after they are 6 days old but by this experiment, 10-12% disease was produced even when 12-13 days old seedlings were utilised for infection (Text fig. 10).



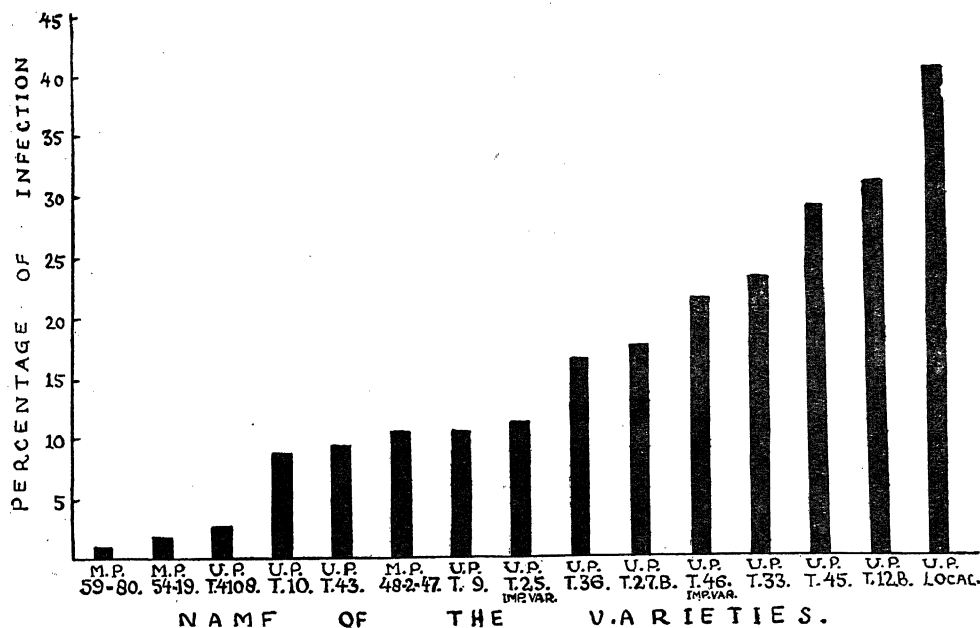
Text Fig. 10.—The effect of age of seedlings of the 'Sawan' on their infection by *U. paradoxa* when grown under normal temperature and under low temperature conditions.

It has been noticed that size of the root and shoot of 8-10 days old 'Sawan' seedlings growing in low temperature, is practically similar to those of 2-3 days old ones under normal conditions chiefly as regards their average length and breadth of the radicle, number and size of the rootlets, the root hairs and the root tip.

#### Varietal Resistance:

There are several varieties of 'Sawan' cultivated in Uttar Pradesh. These are distinguished by the morphological variations in vegetative parts, the seed

character, colour of glumes, the yield of grains and in the time of the first appearance of the floral spike. To study the varietal resistance, 11 varieties obtained from the Economic Botanist to the Government of Uttar Pradesh, Kanpur ; 3 varieties (59-80, 54-19 and 48-2-47) obtained from the Economic Botanist to the Government of Madhya Pradesh, Nagpur and 1 local variety i.e., 15 varieties in all were utilised. The names of the varieties have been maintained as given by the respective suppliers. The externally sterilised seeds were sown in the experimental plots after having been mixed with the smut spores in the usual manner. The results of the experiment are presented in text figure 11.



Text Fig. 11.—The relative infection in the various varieties of 'Sawan' by *U. paradoxa*.

The varieties found most resistant are, 59-80, 54-19 and T-4108 showing less than 1-3% disease. The smutted ears of 48-2-47 and T-36 varieties were marked by the production of hypertrophied grains which in some cases reached to 3-4 times the usual size of a normal healthy grain of 'Sawan' (Plate, Fig. 2). The improved varieties T-25 (Hardoi) and T-46 (Pratabgarh) have been found to be very susceptible to this disease.

#### Discussion :

The infection of *U. paradoxa* in 'Sawan' seedlings has been found to be by the externally seed-borne spores in conformity with Kulkarni's observations, and also by the soil-borne spores. Such is the mode of infection in *U. coicis* (Chowdhury, 1946), *U. crameri* and *U. panici-miliacei* also, the smut parasites of other smaller millets. As against the average infection of 30-40% obtained by the author, Kulkarni (1922) reported 90% infection of 'Sawan' seedlings through the seed-borne spores. The highest percentage of disease ever achieved in the present case is 68%. This may be due to the difference in the varieties used in the two cases because the varietal susceptibility, even under more or less identical conditions, has been found to be different in different varieties collected from various places as is shown

by experiments to study the varietal resistance of 15 varieties including the local one also under Lucknow conditions.

The study of the effect of various nutrients on the spore germination, specially of the smut parasites which germinate and infect the radicle of the host seedlings in the soil, is of considerable importance in as much as it reveals the most suitable or inhibitive medium for its growth (Wolff, 1873 ; Fischer, 1940 ; Ling, 1940 and Tapke, 1948). The spores of *U. paradoxa* have been found to germinate well in nearly all the various media and solutions tried. The only mode of germination observed by the author, is by means of a short or long, branched or unbranched hypha alone, which has been described by Kulkarni (1922) in the distilled water. Although the author's list of the nutrients included the tomato dextrose agar and tomato dextrose (+/- peptone) solutions as well, but the production of the sporidia was not observed even in these, while Kulkarni (1922) reports germination of spores in nutritive tomato broth by the production of promycelium and sporidia which bud freely to produce secondary sporidia also. Dipotassium hydrogen phosphate does not favour easy and profuse germination of the spores and thus acts as an inhibitor.

The cardinal temperatures for *U. paradoxa* spores have been found to be : minimum 15°C, optimum 35°C and maximum 45°C. It is thus clear that the spores can survive the average temperature variations round the year which in this part of Uttar Pradesh, where the work has been carried out, is about 15-18°C in winters and 40-43°C in summers, and bring about new infection in the month of July after the first shower of the monsoons when also the 'Sawan' seeds are sown. As the infection of the seedlings normally occurs within 5 days after the seed sowing, the spores get the optimum temperature (35-38°C) for germination and subsequent infection in the host seedlings in this month.

The temperature induced change of the reactivity differs according to the host-parasite concerned. In general, the resistance of cereals to *Puccinia graminis* diminishes with rising temperature whereas the resistance to *P. glumarum* increases under similar conditions. In the damping-off of *Picea excelsa* caused by *Pythium debaryanum* and *Fusarium bulbigenum*, with the rise in the growth temperature the cell walls become increasingly soft and more readily soluble ; the whole seedling organism expands itself in length and becomes ever less resistant (Roth, 1935). In some cases like onion smut (*Urocystis cepulae*) lower temperature normally tend to depress resistance to attack. According to Lasser (1937) the vernalisation alters the germination of the winter cereals in the seedling stage and at the same time greatly reduces its susceptibility to smut infection. Similarly in 'Sawan' smut caused by *U. paradoxa* the general susceptibility is lowered considerably if the seedlings are subjected to low temperature treatment prior to infection. In the so treated seedlings only 10% infection is obtained as against nearly 40% or more when they grow under natural condition.

Furthermore, the low temperature increases the effective period of infection of *U. paradoxa* in 'Sawan'. It is nearly doubled by this method. It may be due to the delayed formation of the root periderm at low temperature so that the roots remain accessible to infection for a longer period. It has been found to be true for the susceptible varieties of tobacco to the root-rot disease caused by *Thielavia basicola* (Conant, 1927). Similar reaction takes place on the structure of peripheral layers in tubers and rhizomes etc.

The susceptible period is usually very brief in those cases where the smuts secure infection at the seedling stage of their hosts. Like *U. avenae* and *Sphacelotheca sorghi* which infect their hosts only prior to the appearance of the seedling

above the ground and *U. cyanodontis* which cannot infect the grass after 3 or 4 days of its sprouting (Mehta, 1923), *U. paradoxa* also infects the 'Sawan' seedlings before they are 6 days old.

The coincidental germination of the smut spores along with the germination of the host seed affords an opportunity for the parasite to secure infection in the sprouted seedling. Histopathological studies of the artificially infected 'Sawan' seedlings at their various stages of development, have shown the penetration of germ tube through the epiblema of the young root and its development in the tissue inside. The maceration study of the 8 days old seedling revealed the intracellular nature of the hyphae in the cortical and the epidermal cells, while they become mainly intercellular within the growing point of the developing host.

The disease being chiefly caused by the soil- and the seed-borne spores, the fungicides employed for disinfecting the soil or the seeds, prior to sowing, can be tried for this also.

#### **Summary :**

The paper deals with the study of the life history of *Ustilago paradoxa* Syd. and Butl., cause of the smut disease in 'Sawan', *Echinochloa frumentacea* Link.

The smut parasite infects through the radicle of the host seedlings by the externally seed-borne as well as by soil-borne spores.

The optimum susceptibility period of infection in the seedlings is when they are 2 and 3 days old, and there is no infection at all after they are six days old.

The low temperature (8-10°C) treatment in the early stages of development of the 'Sawan' seedlings, prior to the infection experiments, has been found to approximately double the susceptibility period to *U. paradoxa*, but at the same time the general susceptibility of the host plants appears to be considerably decreased by this treatment.

A study of the varietal resistance using 15 varieties in all showed that the Madhya Pradesh variety Nos. 59-80 and 54-10 and Uttar Pradesh variety No. T-4108 (Bara Banki) are the most resistant.

The effect of the solid and liquid media and different concentrations of the individual nutrients on the germination of the spores of *U. paradoxa* has been studied at the optimum temperature of 35°C. Dipotassium hydrogen phosphate is the most inhibitive among all these. The cardinal temperatures have also been determined and the viability period of the brand spores is 4 years, if kept dry. The mode of germination is by means of a branched hypha alone, without any production of the sporidia on it.

#### **Acknowledgements :**

The author wishes to express his sincerest gratitude to Prof. S. N. Das Gupta, D.Sc. (Lond.), F.N.I., for his valuable criticism and helpful guidance during the course of investigation. Thanks are due to Dr. T. R. Mehta, Economic Botanist to the Government of Uttar Pradesh, Kanpur, and the Economic Botanist to the Government of Madhya Pradesh, Nagpur, for kindly supplying the seeds of the various varieties of 'Sawan' for the work and other valuable information.

# **Literature Cited :**

- Butler, E. J. Fungi and disease in plants. Thacker Spink and Co., Calcutta, 1918.
- Chowdhury, S. Some studies on the smut, *Ustilago coicis* Bref. of Job's tears millet. *Jour. Ind. Bot. Soc.*, **25** : 123-130, 1946.
- Conant, G. H. Histological studies of resistance in tobacco to *Thielavia basicola*. *Amer. Jour. Bot.*, **14** : 457-480, 1927.
- Fischer, G. W. Fundamental studies of the stripe smut of grasses (*Ustilago striaeformis*) in the Pacific North West. *Phytopathology*, **30** : 93-118, 1940.
- Garrett, S. D. Brom-thymol blue in aqueous sodium hydroxide as a clearing and staining agent for fungus-infected roots. *Ann. Bot.*, **1** (N. S.) : 563, 1937.
- Kulkarni, G. S. Smut (*Ustilago paradoxa* Syd. and Butl.) on 'Sawan' (*Panicum frumentaceum* Roxb.). *Jour. Ind. Bot. Soc.*, **3** : 10-11, 1922.
- Lasser, E. Der Einfluss von Licht und Jarewisation auf den Befall von Weizen, Hafer und Gerste durch Tilletia, Ustilago und Helminthosporium. (The effect of light and vernalisation on the infection of wheat oats and barley by Tilletia, Ustilago and Helminthosporium). Kuhn-, Arch., **44** : 161-210, 1937. (*Abstr.*, *R. A. M.* **17** : 512, 1938).
- Lavroff, N. N. New and less common smut fungi of the Ustilaginaceae in North and Central Asia. Reprinted from Trav. *Inst. Sci. Biol. Univ. Tomsk.* ii : **35** 1936, (*Abstr. R. A. M.* **16** : 342, 1937).
- Leach, R. Report of the Mycologist. *Ann. Rept. Dept. of Agric. Nyaasaland*, 47-50. 1932, (*Abstr. R. A. M.* **12** : 9-10, 1933).
- Ling, L. Factors affecting spore germination and growth of *Urocystis occulta* in culture. *Phytopathology*, **30** : 579-591, 1940.
- The Ustilaginales of China. *Farlowia*, **4** : 305-351, 1953.
- Mehta, K. C. On the mode of infection and perennation of the smut of 'Doob' (*Cynodon dactylon* Pers.). *Jour. Ind. Bot. Soc.*, **3** : 243-251, 1923.
- Mundkur, B. B. 'Studies in Indian Cereal Smuts' VI. The smuts on Sawan (*Echinochloa frumentacea*). *Ind. Jour. Agri. Sci.* **13** : 631-633, 1943.
- Roth, C. Untersuchungen uber den Wurzelbrand der Fichte (*Picea excelsa* Link). (Studies on the root scorch of the spruce *Picea excelsa* Link), *Phytopathology*, **Z. 8** : 1-110, 1935. (*Abstr. R. A. M.* **14** : 482, 1935).
- Tapke, V. F. Environment and the cereal smuts. *Bot. Rev.*, **14** : 359-412, 1948.
- Wolff, R. Beitrag zur kenntniss der Ustilaginnen. *Bot. zeitung*, **31** : 657-661 ; 673-677 and 689-694, 1873.

## THE DIGESTIVE SYSTEM OF SOME BRITISH AMPHIPODS PART II (DESCRIPTION OF MOUTH PARTS)

By

V. P. AGRAWAL

D. A. V. College, Muzaffarnagar

[Received on November 6, 1962]

Amphipoda forms a large group of the class Crustacea ; 18 species of this order belonging to the different families with diverse feeding habits and habitats have been selected and their feeding habits, feeding appendages and the morphology and physiology of alimentary canal have been studied so as to correlate these different aspects.

In the first paper, (Agrawal, 1962) the feeding appendages of 8 species belonging to 5 different families have been described. In the present paper, the feeding appendages of the remaining 10 species have been discussed in detail. In the subsequent papers, to follow, the alimentary canal of these amphipods with a view to correlate these different aspects will be described and discussed.

### Family—Gammaridae

From the family Gammaridae, 4 species belonging to the genera *Melita*, *Gammarus* and *Marinogammarus* with distinctly diverse habitats have been studied.

*Gammarus chevreuxi* Sexton, (Plate I) a brackish-water species, was first found in June, 1912 by Sexton and Clark, inhabiting the ditches and traversing a low lying salt marsh. It is a small animal about 12 mm long.

The two antennae are well developed. The molar expansion of the mandible is strongly built and bears a large number of tooth-like projections at its outer edge. The incisors are simple.

Both first and second pairs of maxillae are well developed and bear knob-like spines. The masticatory lobe of the maxilliped is well developed and is provided with small blunt spines.

The two lips are normal in structure.

### *Marinogammarus marinus* (Leech)

*Marinogammarus marinus* (Leech) has been recorded by Norman (1889) and Walker (1895) from different localities. It is a raptatory and macrophagous form, feeding entirely on plant material.

A full-grown specimen of *M. marinus* is about 16 mm long. It is a true littoral form being abundant at high water level ; its constant habitat is amongst the dark fuci which grow with much fertility between the tide marks.

The superior antenna of *M. marinus* are provided with a large number of small spines on either side. The mandibles are strongly built to hold and crush the large food particles on which the animal feeds. Its masticatory part has chitinous incisors arranged in two rows. The outer edge of the molar expansion is deeply serrated. The palp of the mandible is very long and bears a large number of long spines. Both upper and lower lips are built on the usual plan.



***Gammarus pulex* Fabricius.**

*Gammarus pulex* Fabricius, is about 16 mm long and is widely distributed in fresh waters of Britain. It lives beneath loose leaves, pieces of wood etc.

The two antennae are clothed with a large number of long spines. The mandible bears sharp and chitinous incisors; its molar expansion is weak. Greatly elongated palp bears a large number of feathered spines distally.

The first maxilla of the two sides show certain differences in their palp. The palp of the right maxilla is more massive than that of the left side and bears a few knob-like structures, while that of the left side bears a few long spines. Both the lobes of the second maxillae bear long plumose spines.

The maxilliped has well developed basal and masticatory lobes which bear a few small thick and a few long feathered spines.

*Melita palmata* Montagu (Plate II) was first studied by Montagu who described it as a species of *Gammarus*. Leech (1813), however, separated it into a separate genus. Reid (1939) has described the animal in some detail.

A full grown animal is about 8 mm long and is commonly found in wet sand and under stones from mid-tide level down to L.W.M. and probably below.

Both pairs of antennae are large with simple spines. The strong mandible bears curved, thickly chitinous incisors and is provided with a well developed molar expansion. The palp of the mandible bears long spines.

The first and second pairs of maxillae are normally built and are provided with simple spines. The masticatory lobe of the maxilliped is well developed and is produced into a large number of small tooth-like and a few long spines. The upper and lower lips are also well developed.

**Family—Lysianassidae**

*Orchomenella nana* Kroyer (Plate III) of the family Lysianassidae is frequently found in moderate depths from 20 to 50 fathoms around the British coast.

The peduncle of the superior antenna is very broad proximally. The large inferior antenna bears only three very small calceoli on its flagellum.

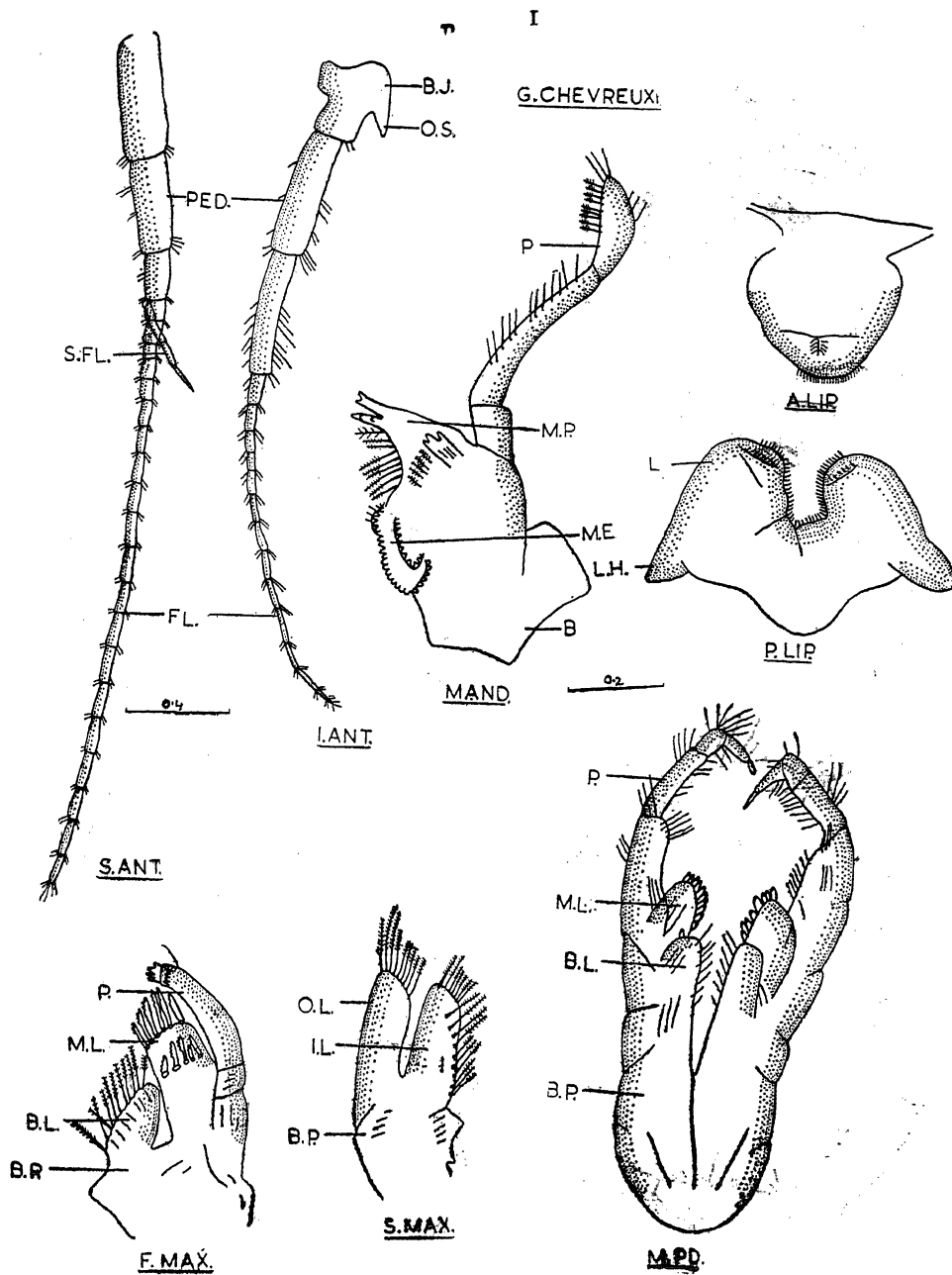
The mandible is comparatively weak in its stature; it bears only one long incisor tooth. The outer edge of the small molar expansion is serrated. The long area between the masticatory part and molar expansion is without any spines.

The first maxilla has a long masticatory lobe. Its basal lobe bears only two spines which are beset with spinules on one side. The palp has a few knob-like structures distally. The second maxilla is simple in structure.

The two basal lobes of either maxillipeds are joined in the middle to form a single flat structure. The inner edge of the large masticatory lobe is deeply serrated. The two lips are of normal size.

**Family—Cheluridae**

*Chelura terebrans* Allman (Plate IV). Allman (1847) founded the family and described the genus *Chelura* in some details. According to Barnard (1950-51), the chelurids are found in uncovered and abandoned limnoriid tunnels; adults always inhabit the outer tiers of the eroded wood, juveniles are found in the deeper tiers. The animal is an injurious xylophagous crustacean. It excavates the saturated wood not only for concealment but also to feed upon it. According to Allman (1847) the excavations of *Chelura* are large and oblique. A full grown specimen of *Chelura* is 6 mm long. The specimens of *Chelura* were obtained from the Marine Biological Station Plymouth.

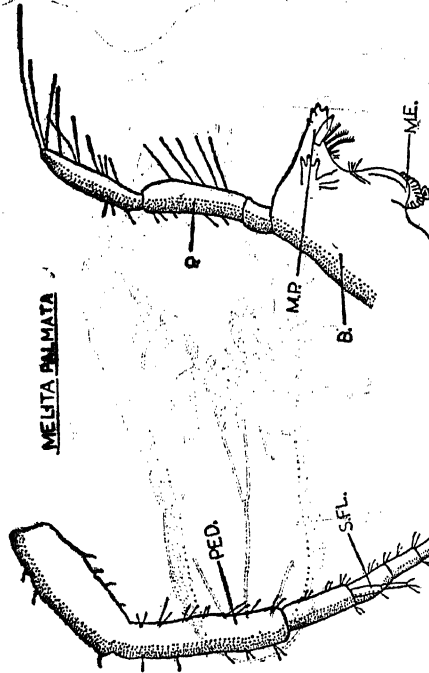


#### ABBREVIATIONS USED

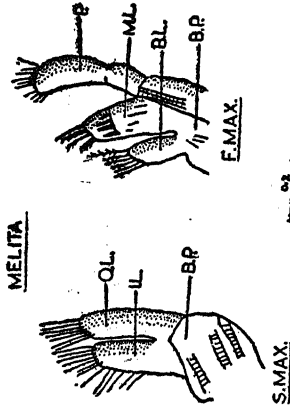
A. Lip., Anterior lip; B., Body; B. J., Basal joint; B. P., Basal part; Cal., Calceolus; FL., Flagellum; F. Max., First maxilla; I. Ant., Inferior antenna; I. L., Inner lobe; L., Lobe; L. H., Lateral horn; Mand, Mandible; M. E., Molar expansion; M. L., Masticatory lobe; M. Pd., Maxilliped; M. P., Maxillary part; O. L., Outer lobe; P., palp; Ped, Peduncle; P. Lip., Posterior lip; S. Ant., Superior antenna; S. Fl., Accessory flagellum; S. Max.; Second maxilla.

N.B.—In all the Figures, the Scale dimension is in mm.

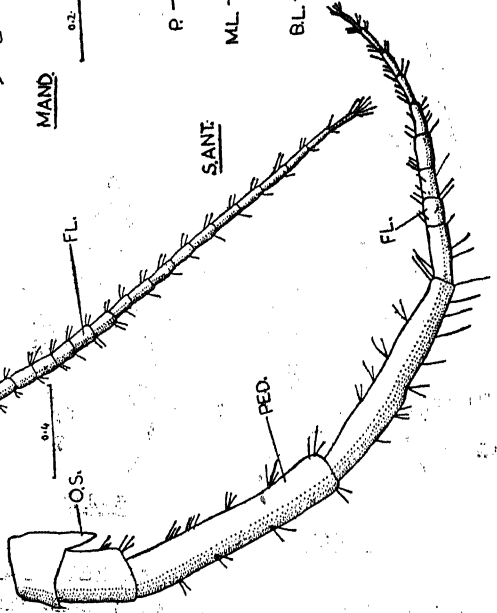
PLATE II



*MELITA PALMATA*



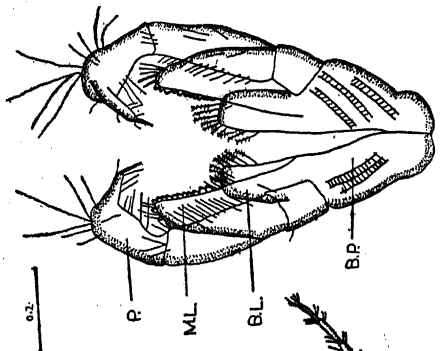
*MELITA*



*MAND.*

*SANT.*

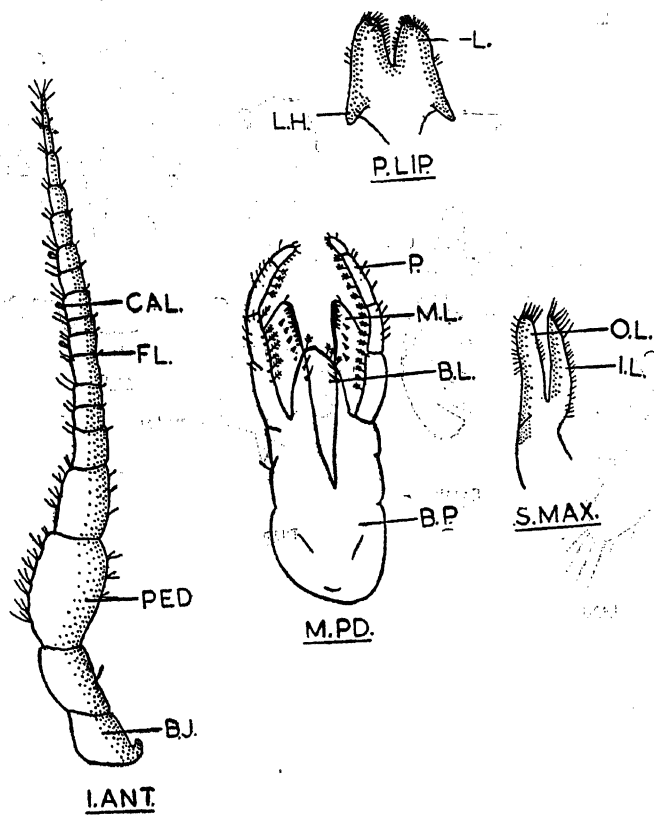
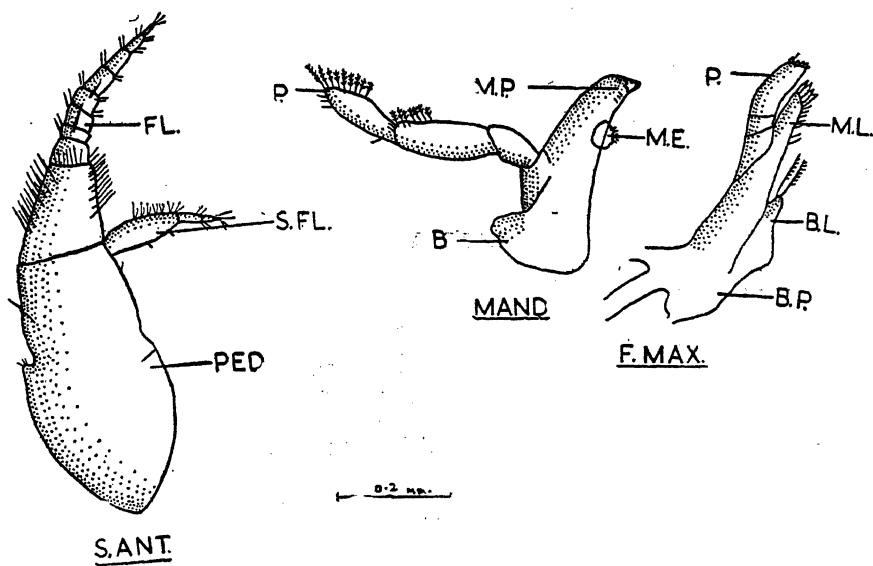
*LANT.*



*M.P.D.*

*ALIP.*

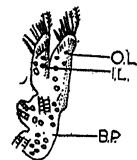
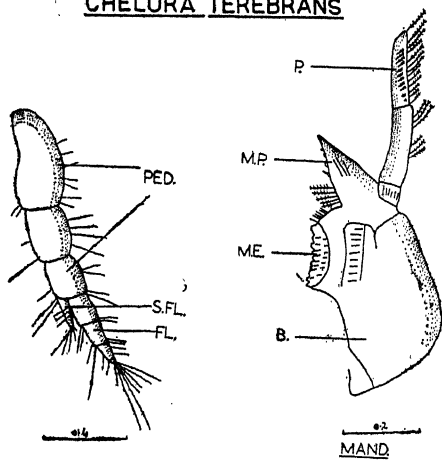
PLATE III



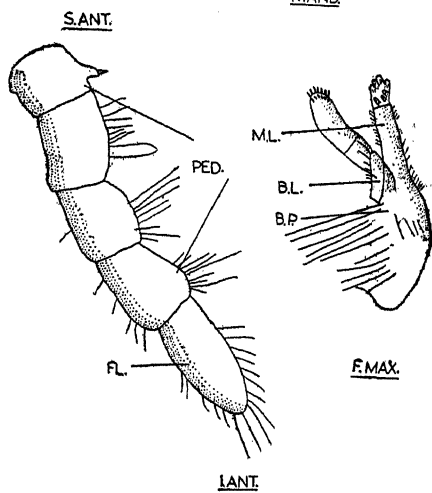
ORCHOMENELLA NANA.

# PLATE IV

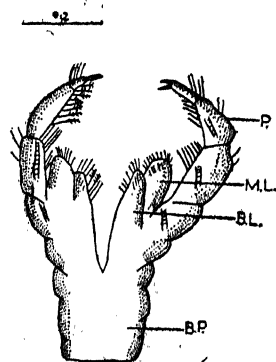
## CHELURA TEREBRANS



S.MAX.



A.U.P.



M.P.D.



P.L.P.

PLATE V

COROPHIUM VOLUTATOR

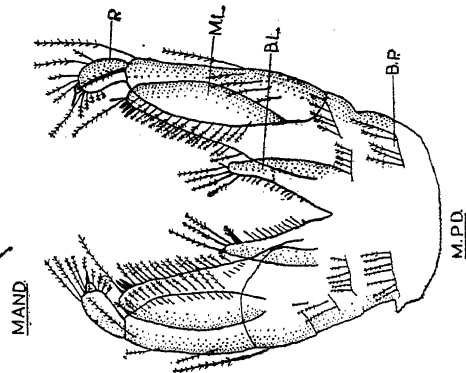
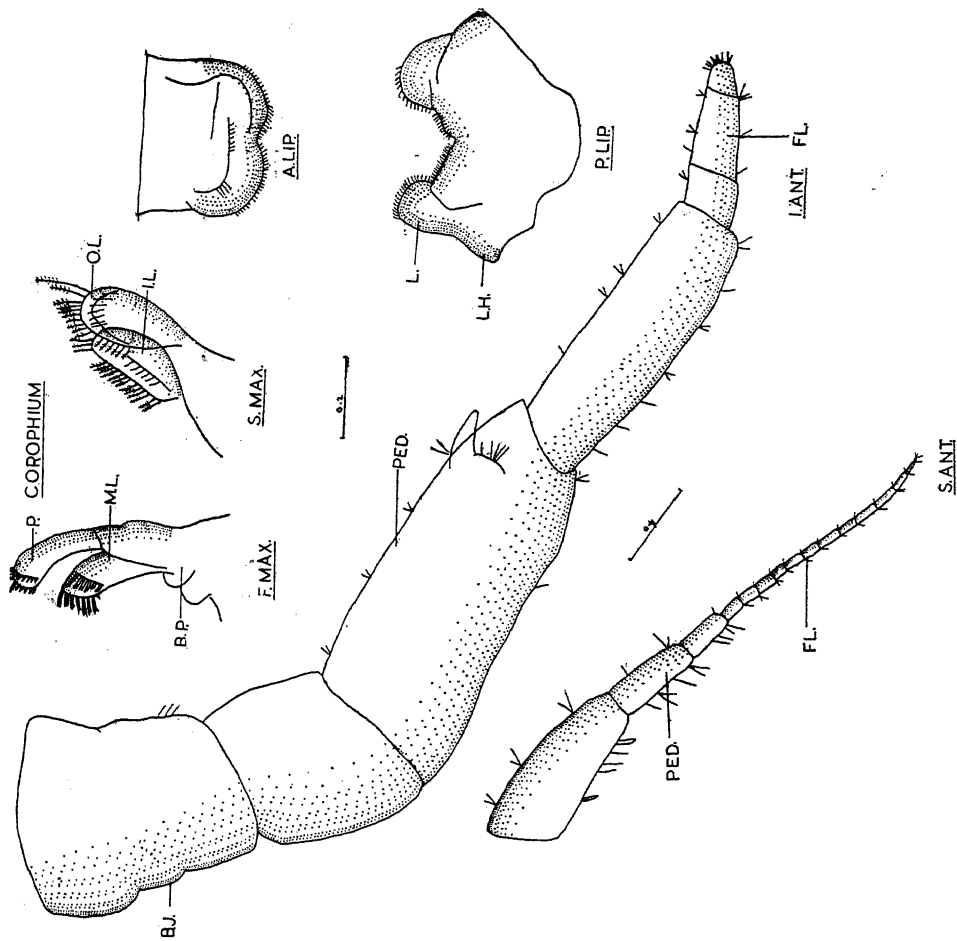
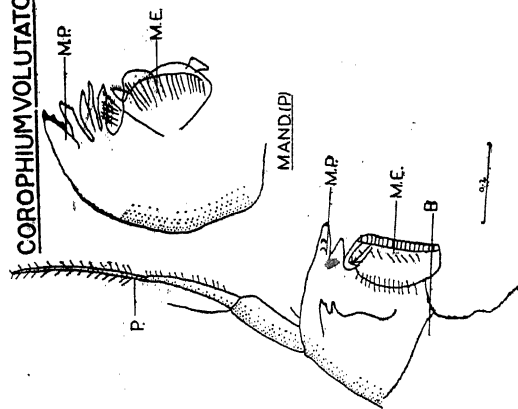


PLATE VI

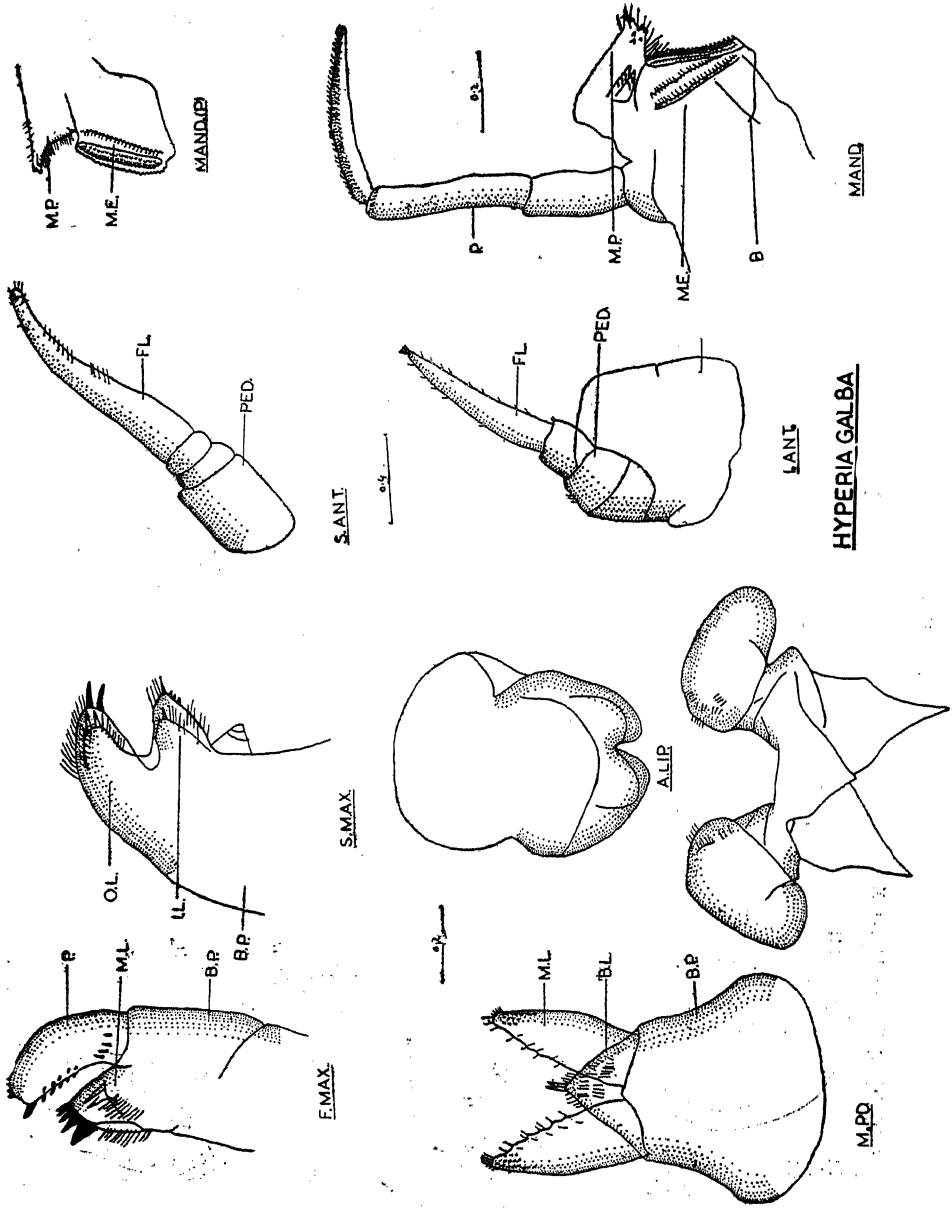


PLATE VII

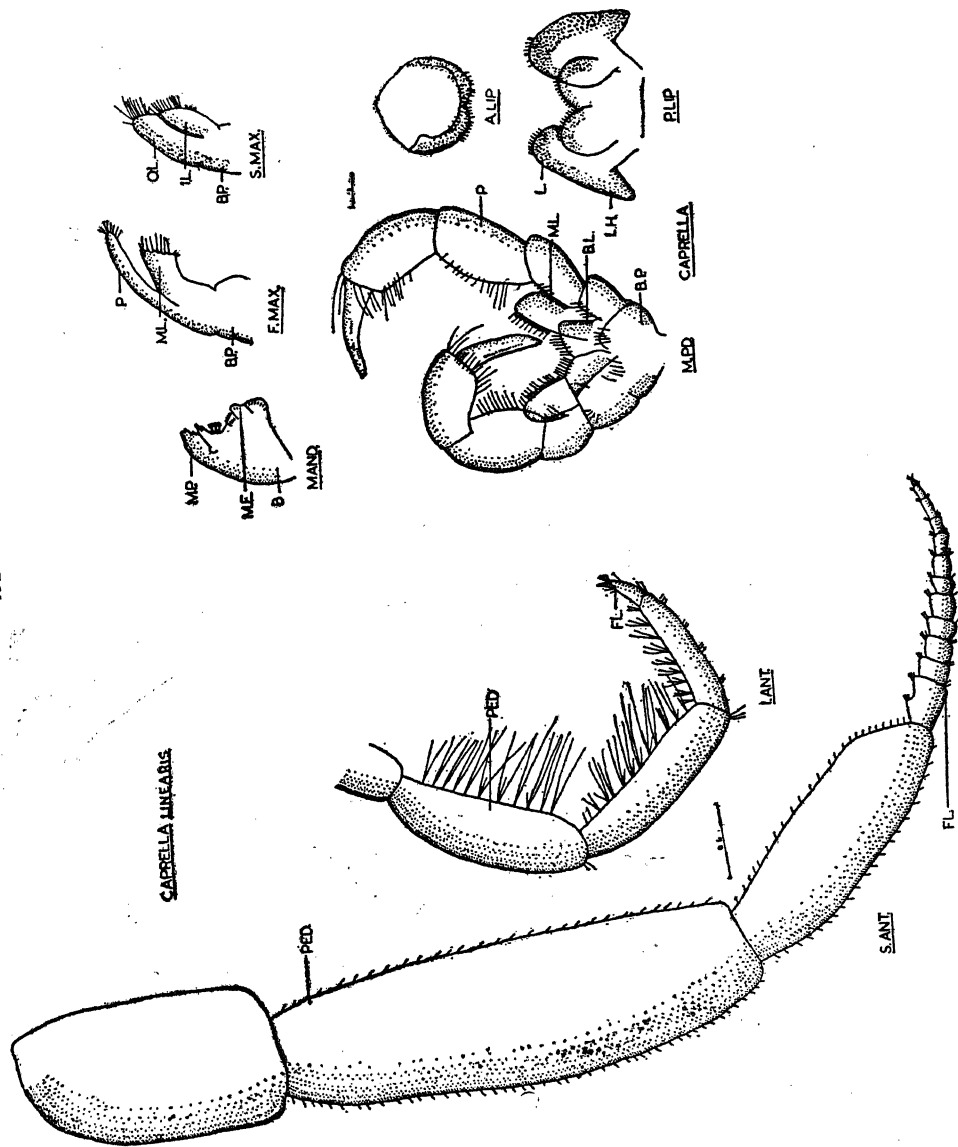
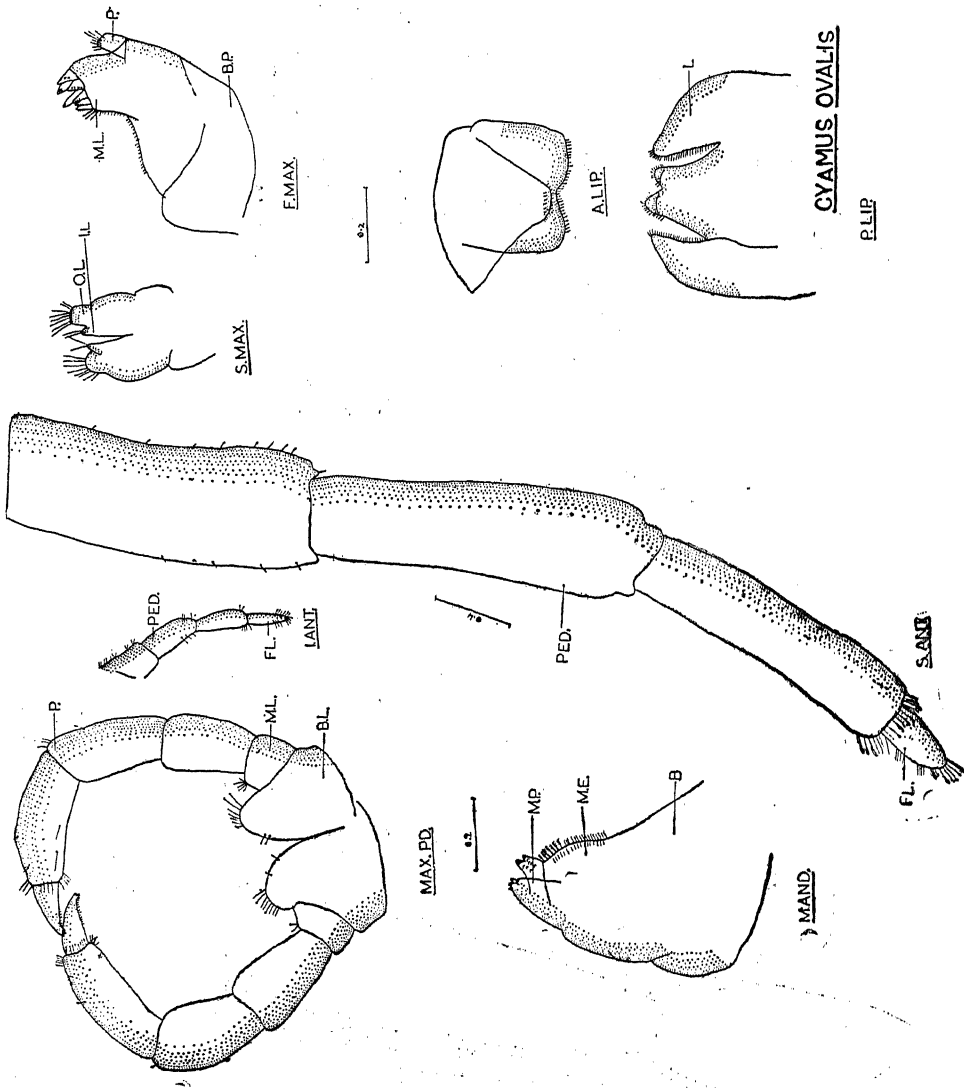




PLATE VIII



The superior antenna is small and bears a small accessory flagellum. The large inferior antenna has a well developed peduncle and an uni-articulate flagellum.

The strong mandible (Plate IV) is thickly chitinous with a few projecting incisors. The molar expansion is also thick with deeply serrated outer edge. The long palp of the mandible bears a large number of feathered spines. The first maxilla of *Chelura* is also strongly built; the masticatory lobe bears a few strong teeth distally. The palp also carries a few small teeth at the top. The second maxilla also bears strong feathered spines. The upper and lower lips are also beset with a large number of small spines.

#### Family—Corophiidae :

*Corophium volutator* Pallas (Plate V). The genus *Corophium* was established by Latreille in 1806 to include the single species *C. longicorne* which was previously described as *Oniscus volutator* Pallas.

*Corophium volutator* Pallas is about 1/3" long, greyish in colour and normally burrows vertically to depth less than 5 inches into the inter-tidal mud. In most cases the burrows are U-shaped but they might be oblique with an opening on either side. Sometimes the number may be so large that the surface of the mud may be punctured throughout.

The feeding of *Corophium* has been studied by various authors. Bate and Westwood (1855) state that they attack annelids and mollusks, the only other animals occurring in the same mud habitat. According to Hart (1930), the animal feeds on organic detritus, mainly vegetable, present in its natural habitat. It seems to feed entirely by selecting particles from the mud in which it lives. According to Hunt (1925), it is a true selective deposit feeder, though when in its burrow, the current produced by the beating of the pleopods brings small particles in suspension so that, in addition, there is a filter feeding mechanism. Crawford (1937) also described *Corophium* as principally a selective feeder and that it also consumes a small proportion of suspended particles.

The superior antenna is small and does not have an accessory flagellum; the inferior antenna is much larger with a very large peduncle and a small tri-partite flagellum.

The mandible is strongly built and bears a few hooked incisors. The molar expansion of the mandible bulges out as a large prominence; its outer surface is deeply serrated. The palp is very long and narrow and is provided with a large number of small spines.

The basal lobe of the first maxilla is very small and rudimentary. Both masticatory lobe and palp are well developed and beset with small tooth-like spines distally. The second maxilla is large and has a large number of feathered spines.

The masticatory lobe of the maxilliped is exceptionally large. Its palp is also large. The entire surface of the maxilliped is clothed with a large number of long feathered spines. Both the lips are also well developed and bear a large number of small spines distally.

#### Family—Hyperiidæ

*Hyperia galba* Montagu (Plate VI) has been studied as the representative of the family Hyperiidæ. Gould (1841) and Goose (1853) have found the animal inhabiting the sub-genital pouches of medusae. Hollowday (1948), has also assigned the

same habitat. According to Edward (1868), they are parasitic in their habit and live in the gill cavities of medusae, though they are also occasionally found on fishes.

However, it is not certain if it is a true parasite as it can survive independently, outside the body of medusae. A few specimens of *Hyperia* were procured from Marine Biological Station Plymouth. A full grown specimen is about 10 mm long and is sandy in colour.

The peduncle of the small but stout superior antenna is free of any spines; the long flagellum is uni-articulate and bears very small and simple spines. The inferior antenna is also small. The basal joint of the peduncle is very broad; the uni-articulate flagellum is provided with very small spines.

The mandible is strongly built; its masticatory part has a large number of small chitinous incisors. The large molar expansion of the mandible bears many rows of tooth-like structures. On its outer side is present a row of thick spines projecting outwards. A few very fine spines or bristles are present in between the incisors and molar expansion. The tri-articulate palp is beset with only a few small spines distally.

The first maxilla of *H. galba* is without a basal lobe. The masticatory lobe is also small and is provided with a few large conical teeth. The uni-articulate palp also bears a few small tooth-like structures. Each lobe of the second maxilla bears two or three strong tooth-like structures and a few long spines.

The maxilliped is characterised by the absence of palp. The basal lobes of the two maxillipeds are united to form a flat, triangular structure. The masticatory lobe is well developed and bears a few small spines.

The large anterior lip is free of any spines. The posterior lip is exceptionally large and is divided into two widely separated lobes which possess a few rows of spines at their inner distal prominences.

#### **Family—Caprellidae**

Family Caprellidae of the sub order Caprellidea is the most specialised group of amphipods so far as its shape is concerned. The caprellids can be easily recognised by their long slender bodies. Several observers have maintained that they feed on hydroids and algae. According to Harrison (1940) they feed on copepods and nauplii. In the laboratory, they can be fed on a variety of materials such as meat, mashed up crustacea and bread soaked in meat juice.

*Caprella linearis* (L) (Plate VII) are found below the low water mark, attached by their pleopods to the less dense forms of hydroids, algae, buoys, floating wreckage etc. *Caprella* were collected from Whitstable sea coast.

The superior antenna is very large with a very long peduncle and a small multi-articulate flagellum. Both peduncle and flagellum are provided with small and simple spines. There is a single flagellum provided with small and simple spines. There is let it stand as such flagellum. The inferior antenna is small with a comparatively large peduncle and a small uni-articulate flagellum. The peduncle is produced into a large number of long spines.

The mandible is very small and is without a palp. The cutting edge bears a few small incisors. The molar expansion is poorly developed. Three feathered spines are present between the incisors and molar expansion.

The first maxilla is without a basal lobe. The palp is long and narrow and bears a few spines distally. The masticatory lobe is well developed and is

prouduced distally into a few feathered spines. Both the lobes of the second maxilla are provided with a few long fearthred spines.

The maxilliped is very large while its basal lobe is small. The large masticatory lobe bears a few strong teeth and a large number of long spines. The palp is very large and bears long spines. The two lips are built on the usual plan.

#### Family—Cyamidae

*Cyamus*, the whale louse of the family Cyamidae was placed in the order Isopoda by Linnaeus because of its much flattened body. Latreille (1805) placed the genus with the *Gammrii* in the order Amphipoda, but he subsequently united it with *Caprella* to form a distinct order. Barnard (1932) has given a brief account of the genus. Iwasa (1934) has descrided a few species of the genus in some detail.

The body of *Cyamus ovalis* (L) (Plate VIII) is flattened dorso-ventrally. Normally the animal is about 9 mm long. It is an ectoparasite and is found attached to the skin of the whales by means of its strong appendages. The specmens of *Cyamus* were obtained from the Natural History Museum, London.

The superior antenna is exceedingly large with a massive peduncle and a small uni-articulate flagellum which bears a few small spines. The inferior antenna is small and rudimentary and has a small uni-articulate flagellum.

The mandible is poorly developed and pyramidal in shape. The masticatory part has two sets of strong chitinous incisors. The molar expansion is weakly developed. Between the incisors and the molar part of the mandible are a few feathered spines. The palp is absent.

The first maxilla of *Cyamus* has no basal lobe ; the strong masticatory lobe has seven denticules and few fine spines. The small uni-articulate palp bears, a few small spines distally. The second maxilla has both outer and inner lobes. The two inner lobes of the second maxilla are united in the middle. The inner lobe of each side has a small knob-like projection carrying two long spines distally.

The two maxillipeds are joined in the middle to form the floor of the mouth. It has a large basal lobe and a small masticatory lobe. The palp is very large and has four joints ; the last joint is claw-shaped and is bifurcated at its tip. The palp on its different joints, carry fine tooth-like structures arranged in rows.

The anterior lip has a prominent central thickening which also bears a few small spines. The posterior lip is peculiar in shape and consists of two paired lobes. The inner ones are fused together to form a single bifurcated structure bearing small spines distally bent. The outer lobes are inwards and are clothed with small spines at thier inner surfaces.

#### Summary :

In this paper, the feeding appendages of ten species of amphipods represent- ing seven families have been described.

It has been found that in the macrophagous forms, such as *Marinogammarus marinus*, *Gammarus pulex*, *G. chevreuxi*, *Melita palmata* and *Caprella linearis*, the feeding appendages especially the mandibles are very strongly built to crush the large, hard food particles.

In *Orchomenella nana* and *Corophium volutator*, which are pricipally microphagous feeders, the mandibles are comparatively weak. *Chelura terebrans* which

is a xylophagous form has very strong mandible to scrape off the food from the wood in which it lives. *Hyperia galba* lives in the sub-genital pouches of the medusae and has very strong mandibles.

While *Cyamus* is an ectoparasite on whales and has weak mandibles.

#### Acknowledgement :

The author is indebted to Prof. J. E. Smith, Sc.D., F.R.S. of London University under whose supervision this work was conducted.

#### References :

- Allman, G. L. On *Chelura*, an Amphipodous Crustacean destructive to submarine timber works. *Ann. Mag. nat. Hist. Ser. I*, **19** : 361-370, 1847.
- Barnard, K. H. Amphipoda. *Discovery Reports Cambridge*, **5** : 1-326, 1932.
- Barnard, J. L. The role of *Chelura* in the destruction of timber. 1951. Marine Borer Conf. U. S. Navy Civ. Eng. Res. Eval. Lab. Port Hueneme Calif. May, 10-12, 1951 Rept.
- Bate, Sp. and. Westwood, J. O. A History of the British Sessile-eyed Crustacea, Vol. I and II, 1955-65.
- Crawford, G. I. A review of the Amphipod genus *Corophium* with notes on the British species. *J. mar. biol. Ass. U. K.* **21** : 589-630, 1937.
- Edward, Th. Stray notes on some of the smaller Crustaceans. Note II. On the habits of the Hyperiidæ. *J. Linn. Soc.* **2** : 166, 1868.
- Goose, P. H. A Naturalist's Rambles on the Devonshire Coast, London, *J. van. Voorst*, 367-68, 1853.
- Gould, A. A. Report on an investigation of Massachusetts. Cambridge, U. S. I. Zoological and Botanical Survey of Mass, 1841.
- Harrison, R. J. On the biology of the Caprellidae. Growth and moulting of *Pseudoprotella phasma* Montagu. *J. mar. biol. Ass. U. K.* **24** : 483-493, 1940.
- Hart, T. J. Preliminary notes on the bionomics of the amphipod *Corophium volutator*. *J. mar. biol. Ass. U. K.* **16** : 761-789, 1930.
- Hollowday, E. D. On the commensal relationship between the amphipod *Hyperia galba* and the scyphomedusa *Rhizostoma pulmo*. *J. Ouekett. micr. cl. (4)* **11** : 187-190, 1948.
- Hunt, O. D. The food of the Bottom Fauna of the Plymouth fishing ground. *J. mar. biol. Ass. U. K.* **13** : 560-599, 1925.
- Iwasa, M. Two species of whale lice (Amphipoda, Cyamidae) parasite on a whale. *J. Fac. Sci. Hokkaido Zool.* **3** : 33-39, 1934.
- Latereille, P. A. Histoire generale et particuliere des Crustaces et des Insectes. (Suites a Buffon, Paris), 1867.
- Leech, W. E. Crustaceology. (Brewster's Edinburgh Encycop. Edinb.), 1813.
- Norman, A. M. Notes on the British Amphipoda. Gammaridae. *J. R. Micr. Soc.*, **19** : 511, 1889.
- Reid, D. M. Melita Sp. (Crustacea, Amphipoda) *Ann. Mag. nat Hist. (II)* **4** : 278-281, 1939.
- Sexton, E. W. Description of a new species of brackish water *Gammarus* (*G. chevreuxi*). *J. mar. biol. Ass. U. K.* **9** : 542-545, 1913.
- Walker, A. O. Revision of the amphipoda of the L. M. B. C. district. *Tr. Liverp. Biol.*, **9** : 287-320, 1895.

# THE LOCALIZATION OF VERNALIZATION INFLUENCES

By

S. C. CHAKRAVARTI

Government Degree College, Mhow (M. P.)

[Received on February 19, 1963]

In a previous communication Chakravarti (1951) demonstrated that shoots developed from axillary buds in vernalized mustard plants flowered earlier than their corresponding counter-parts on the control ones. From this he concluded that the seat of vernalization changes is the apical meristem from which axillary ones are derived. Similar conclusions could also be arrived at from the data presented by Sen and Chakravarti (1946) showing that an earliness in ear emergence is maintained by different tillers on the vernalized wheat plants when compared with their counterparts on the control. Purvis (1940) while working with rye recorded that the entire plant regenerated from the apical meristem of the treated embryo gave vernalization response. Similar was the observation of Sen and Verma (1957) with mustard. Recently Wellensiek (1961, 1962) working with leaf and root cuttings of *Lunaria biennis* concluded that vernalization occurs in the cells in the process of division.

In contrast to the above, Schwabe (1950) working with vernalized *Chrysanthemum* found that little or none of the effect of vernalization is normally carried into the young basal shoots produced on the old stems of the vernalized plants. Efeikin (1958) working with fodder beet came to the conclusion that the stimulus induced by low temperature and necessary for flowering is not restricted to the local changes in the meristem.

TABLE 1

Effect of inducing primary to quarternary branches in vernalized and control plants of *Cicer arietinum* L., T. 87 on flowering.

Date of sowing 5.11.60

V. 45 days

Number of plants are given within brackets

Nature of shoot	Vernalized	Control	Earliness
Main axis undisturbed	49.3 (12)	71.4 (13)	22.1*
Primary branches	50.4 (15)	69.3 (15)	19.9*
Secondary branches	61.1 (11)	74.5 (12)	13.4*
Tertiary branches	64.7 (8)	75.1 (8)	12.4*
Quarternary branches	66.9 (12)	75.8 (6)	8.9**

\*Significant at 1% level

\*\* Significant at 5% level

With a view to collect further data on the subject an experiment was carried out with *Cicer arietinum* L., T. 87 in flower pots. Shortly after the germination of vernalized and control plants, they were decapitated above the cotyledonary node

to induce the growth of axillary buds, only one of which was allowed to develop. Four pots containing about 16 such plants were set aside, while in the rest, the tips of the axillary branches were removed after the formation of the first leaf. This process was repeated till there were four pots for each of the series of plants having primary, secondary, tertiary and quaternary branches. The date of the opening of the first flower on them in both control and vernalized plants was recorded and the data are presented in table 1.

It would be seen from the above table that there has been a significant earliness in flowering even on quaternary branches, which were derived from the repeated division of the original meristem at the axil of the cotyledonary leaf receiving the direct stimulus at the time of vernalization. The progressive diminution in the earliness induced from the main axis onwards compare favourably with that recorded in mustard by Chakravarti (1951) and seems to be due to the increase in the natural cold during the development of these branches.

Schwabe's (1950) failure to record early flowering in basal shoots produced on old stems of vernalized plants of *Chrysanthemum*, which have already flowered could be ascribed to the fact that after development of flowers, the stimulus resulting from low temperature treatment gets used up. A similar case was recorded by Chakravarti (1951), where he found that there was no significant difference in the flowering of shoots produced on the vernalized and control plants after they had flowered. Efeikin's (1958) conclusion that transition of plants from the vegetative to the reproductive phase depends not on restricted local changes in the meristem but on the condition of the plant organ as a whole does not seem to be justified. In fodder beet, the apical meristem being removed could not play any part in the transmission of the stimuli but the adventitious shoots, developed on the cut stems, were derived from resting cells, which in their turn had their origin from the meristem that experienced the winter cold. The present study clearly points out that any kind of meristem apical or axillary, with limited mitotic division even, is capable of receiving and retaining the vernalization stimulus which increases autocatalytically with every division of the cells and gets spent up only when the flowers have been formed.

#### Literature Cited :

- Chakravarti, S. C. Seat of vernalization changes during the ontogeny of mustard. *Sci. and Cult.*, **17** : 35-36, 1951.
- Efeikin, A. K. The localization of thermal and photoperiodic influences which induce flowering in plants. *Doklady Akad. Nauk, S. S. S. R.* **117** : 1068-9, 1958.
- Purvis, O. N. Vernalization of fragments of embryo tissue. *Nature*, **145** : 462, 1940.
- Sen, B. and Chakravarti, S. C. Annual Report, I. C. A. R., New Delhi, Scheme on Cytology and Plant Physiology, 1946.
- Sen, B. and Verma, G. Cultivation of tissue fragments of embryo and seedlings of control and vernalized seeds of mustard T. 102. *Proc. Ind. Sci. Cong.*, **3** : 279, 1957.
- Schwabe, W. W. Factors controlling flowering in the *Chrysanthemum*. I. The effects of photoperiod and temporary chilling. *J. Expt. Bot.*, **1** : 329-343, 1950.
- Wellensiek, S. J. Leaf vernalization. *Nature*, **192** : 1097-1098, 1961.
- Dividing cells as the locus for vernalization. *Nature*, **195** : 307-308, 1962.

# THE HEART OF INDIAN CUCHIA EEL, *AMPHIPNOUS CUCHIA* (HAM.)

By

H. S. CHAUDHRY and A. B. DAS  
University of Gorakhpur, Gorakhpur

[Received on 17th February, 1962]

## Introduction :

The *Amphipnous cuchia* (Ham.), a common Indian mud-eel belonging to family Amphipnoidae and order Symbranchiformes (Berg, 1947), is well known for its amphibious habit. It possesses the remarkable capacity of aerial respiration (Das, 1928). In spite of this, surprisingly enough, it did not attract much attention of the morphologists and physiologists. However, some fragmentary work on the vascular system of *Monopterus javanensis*, a member of the allied family Symbranchidae, has been done by Muller (1939), Hyrtl (1859) and Volz (1906). Recently Wu and Liu (1943) have given a more comprehensive account as a result of the reinvestigation of the problem. Parsons (1929) in his study of the conus arteriosus of teleosts has also made a brief reference of the bulb of *Symbranchus*, another allied form. A perusal of the literature reveals the lack of any description of the cardiac anatomy of the Indian Cuchia Eel, which prompted the present authors to initiate this study.

## Observations :

The heart of *Amphipnous cuchia* is enclosed in a thin tough pericardial membrane. It is situated in the anterior part of the body cavity approximately 1/5 of the body length from the snout, nearly 2-3 inches behind the last gill-slits. The ventral aorta is consequently unusually long. The general shape of the heart is tubular, measuring from 1½-2 inches in length. It is widest in the middle and gradually tapers towards the two ends. The heart of *Amphipnous*, like the typical teleostean heart as described by Goodrich (1930), consists of four distinct regions.

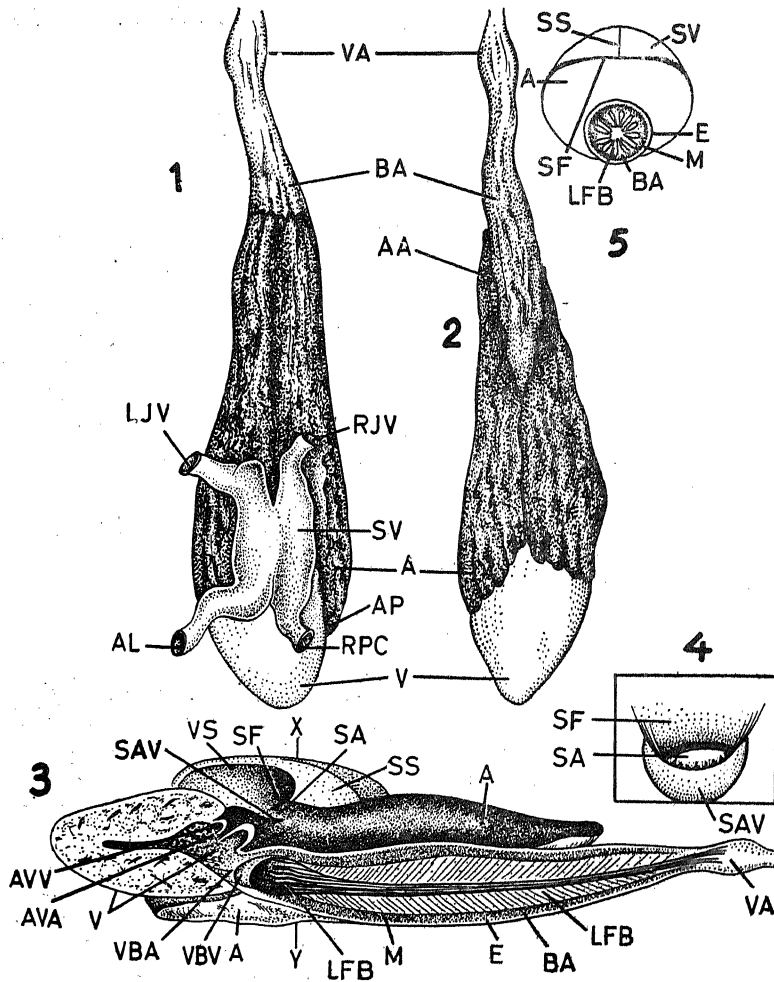
*Sinus Venosus*.—The sinus venosus is a squarish thin-walled chamber. Its dorsal wall is somewhat pigmented. Anteriorly the sinus venosus receives a right and a left jugular vein. Towards its posterior end joins an azygos hepatic vein on the left side, and an interrenal or the right postcardinal vein on the right side. The ductus Cuvieri of both sides are indistinguishable (fig. 1).

The sinuatrial aperture is oval and is transversely disposed in the posterior dorsal wall of the auricle. It is bordered all round by a thick muscular band. Projecting from the posterior lip of this aperture into the atrium is present a single membranous flap-like sinuatrial valve. The anterior margin of the valve is finely frilled, whereas its two ends are extended forwards which get attached to the roof of the auricle. The valve is capable of overlapping the anterior margin of the sinuatrial aperture and thus completely guards it (fig. 4).

Another peculiar feature in the sinus venosus of *Amphipnous* is the presence of a thin median longitudinal fold-like structure on the inner surface of its dorsal wall. Anteriorly the ventral margin of this fold is completely attached to the floor of the sinus upto the anterior lip of the sinuatrial aperture. While posteriorly, behind the hinder lip, it is quite free. It gradually gets reduced backwards and finally disappears just in front of the hinder limit of the sinus. The sinus venosus thus can be differentiated into two portions : An anterior region divided completely into two longitudinal chambers by this flap-like septum, each half receiving



PLATE I



EXPLANATION OF FIGURES AND LETTERING

Figures showing the structure of the heart of *Amphipnous cuchia*. 1. Dorsal view; 2. Ventral view; 3. Median longitudinal section; 4. Sinu-auricular aperture with valve from ventral side; 5. C. S. of heart at XY showing sinus septum dividing the anterior part of Sinus venosus.

A. auricle; AA. anterior arm of auricle; AP. Posterior arm of auricle; AL. azygos hepatic vein; AVA. auriculo-ventricular aperture; AVV. auriculo-ventricular valve; BA. Bulbous arteriosus; E. epicardium; LJV. Left jugular vein; LFB. longitudinal fold of bulbous; M. mesocardium; RJV. right jugular vein; RPC. right post-cardinal; SA. sinu-atrial aperture; SAV. sinu-atrial valve; SF. sinus floor; SS. sinus-septum; SV. sinus venosus; V. ventricle; VA. ventral aorta; VBA. ventriculo-bulbular aperture; VBV. ventriculo-bulbular valve.

the jugular vein of the corresponding side, and a posterior region where due to incomplete and shallow flap the cavity remains undivided (figs. 3, 5).

**Atrium.**—The atrium is a large more or less H-shaped thin-walled chamber lying ventral to the sinus venosus. Its antero-lateral arms are produced forwards and backwards enveloping tightly the entire length of the underlying bulbus arteriosus. Posteriorly the atrium is produced into two similar lateral horns, embracing the dorsolateral sides of the ventricle (figs. 1, 2). The atrial wall is perfectly smooth internally and does not show the characteristic musculi pectinati as reported in most other teleosts. The auriculo-ventricular opening is a transverse slit on the posterior wall of the auricle. It is guarded by a dorsal and a ventral large cup-shaped auriculoventricular valves. Their free margins are attached to the ventricular wall by a number of chordae tendineae (fig. 3).

**Ventricle.**—It is a thick-walled muscular conical chamber with its posterior apex bent a little to the right side of the animal. The muscles of the ventricle are loosely decussated on its inner surface which bears a number of blood pits. The lumen of the ventricle is greatly reduced and communicates with the cavity of the bulbus through a ventriculobulbular aperture situated a little anterior and ventral to the atrioventricular opening. The ventriculobulbular aperture is guarded by two laterally placed semilunar ventriculobulbular valves, separating the ventricle from the bulbus. This single pair of valves, as discussed by Goodrich (1930), represent the reduced Conus Arteriosus of *Amphipnous*, exhibiting a typical teleostean feature (fig. 3).

**Bulbus Arteriosus.**—It is a long tubular structure partially visible from the ventral side between the ventrolateral wings of the auricle. It has a fairly thick elastic wall. Its inner surface is trabeculate, showing eight to twelve longitudinal folds extending from the aortic to the ventricular end of the bulbus. These folds appear to be united with each other at the base (fig. 3, 5).

#### Discussion :

The study of the heart in this amphipnid teleost reveals the following interesting morphological features, most of which can be explained due to the ophidian elongated body-shape and adaptation to the burrowing habit :

(1) The posterior situation of the heart, far behind the pectoral region as seen in *Amphipnous*, has also been reported in Symbranchidae and Apodes in general, but rarely in other fishes (Owen, 1846). The same condition has been described in *Monopterus* by Wu and Liu (1943).

(2) The elongated tubular shape of the heart and the extra development of the auricular appendages in *Amphipnous* recall similar features described in the heart of *Monopterus* (Wu and Liu, 1943) and to some extent in the heart of *Anguilla* (McWilliam, 1885 and Mott, 1950). However, the anterior auricular horns in *Amphipnous* are not so much developed as in *Monopterus*.

(3) The great reduction and obscurity of the ductus Cuvieri, as found in this fish, also characterize the Blood-Vascular system of *Anguilla* (Mott, 1950) and *Monopterus* (Wu and Liu, 1943).

(4) The single hepatic vein opening into the left side of the posterior wall of the sinus venosus and the single right-interrenal or postcardinal vein in this teleost are similar to those described in the allied form, *Monopterus* (Wu and Liu, 1943).

(5) The presence of a well developed flap-like partition in the anterior region of the sinus cavity is very peculiar. A somewhat similar, but shallow partition

has been reported in *Monopterus* by Wu and Liu (1943). But they have offered no explanation regarding its functional significance. However, in *Amphipnous* it seems to play an important role. The heart of *Amphipnous*, which is situated far behind the pectoral region unsupported by any ventral skeletal structure, is subjected to the pressure of its body weight when the fish is outside water in mud or burrow. Under such condition the fold prevents the thin roof of the sinus to come in close contact with the floor and thus protect the sinus from complete collapse during systolic activity. Further, Prakash (1953) has demonstrated the presence of atrioventricular muscle fibres in *Heteropneustes fossilis* and has attributed to them the function of conducting contraction stimuli from atrium to ventricle. According to Prosser (1962), the heart beat in adult fish normally originates in the sinus venosus. Possibly, therefore, the sinus flap of *Amphipnous cuchia* may have to do something with the conduction of stimuli from sinus to atrium.

(6) In *Monopterus*, Wu and Liu (1943) have described two sinuatrial valves continuous with each other. While in *Amphipnous* there is a single valve like *Heteropneustes fossilis*, which is said to approach the dipnoan condition in this respect (Prakash, 1953).

(7) The inner wall of the bulbus in *Amphipnous* is trabeculate similar to that of *Symbranchus* figured by Parsons (1929). But in *Monopterus*, Wu and Liu (1943) observed only a few wrinkles in the middle portion of the bulbus. Gegenbaur (1891) opined that the teleosts indicate a trend from the ridged to a smooth condition of the inner surface of the bulbus. According to this hypothesis, the absence of trabeculae in the bulbus of *Monopterus* is a specialised feature.

#### References :

- Berg, L. S. Classification of fishes both recent and fossil. Mich, 1947.  
 Das, B. K. The bionomics of certain airbreathing fishes of India together with an account of the development of the airbreathing organs. *Phil. Trans. Roy. Soc. Lond.* CXXVI (B) : 183-221, 1928.  
 Gegenbaur, C. Uber den conus arteriosus der Fische. *Morph. Jahrb.*, XVII : 1891.  
 Goodrich, E. S. Studies on the Structure and Development of Vertebrates. London, 1930.  
 Hyrtl, J. Uber den 'Amphibien-Kreislauf von *Amphipnous* and *Monopterus*. Denkschr. Akad. Wiss. Wien., 14 : 39-48, 1858.  
 McWilliam, J. A. On the structure and rhythm of the heart in fishes with special reference to the heart of eel. *J. Physiol.*, 6 : 192-195, 1885.  
 Mott, J. C. The gross anatomy of the blood vascular system of the eel, *Anguilla anguilla*. *Proc. Zool. Soc. Lond.*, 120 (III) : 503-518, 1950.  
 Owen, R. Lectures on the comparative anatomy and physiology of the vertebrate animals. Part I, Fishes, 1846.  
 Parsons, C. W. The Conus Artriosus in Fishes. *Quart. Jour. Micr. Soc.*, 73 (1) : 145-176, 1929.  
 Prakash, R. The heart of the common Indian Catfish, *Heteropneustes fossilis* (Bloch), with special reference to the conducting system. *Proc. Zool. Soc. Bengal.* 6 (2) : 113-119, 1953.  
 Prosser, C. L. and Brown, F. A. Comparative Animal Physiology. *Phil.*, 1962.  
 Volz, W. Der Circulation und Respirations appart von *Monopterus javanensis*. *Lac. Zool. Jahrb.*, 23 : 163-186, 1906.  
 Wu, H. W. and Liu, C. K. The Blood Vascular system of *Monopterus javanensis*, an airbreathing fish. *Sinensis, Nanking*, 14 : 61-97, 1943.

THE BEHAVIOUR OF THE INTEGUMENTARY TAPETUM IN THE  
OVULES CONTAINING DEGENERATING GAMETOPHYTES IN  
*UTRICULARIA FLEXUOSA* VAHL.

By

REAYAT KHAN

*Department of Botany, Aligarh Muslim University, Aligarh, India*

[Received on December 1, 1962]

**Introduction :**

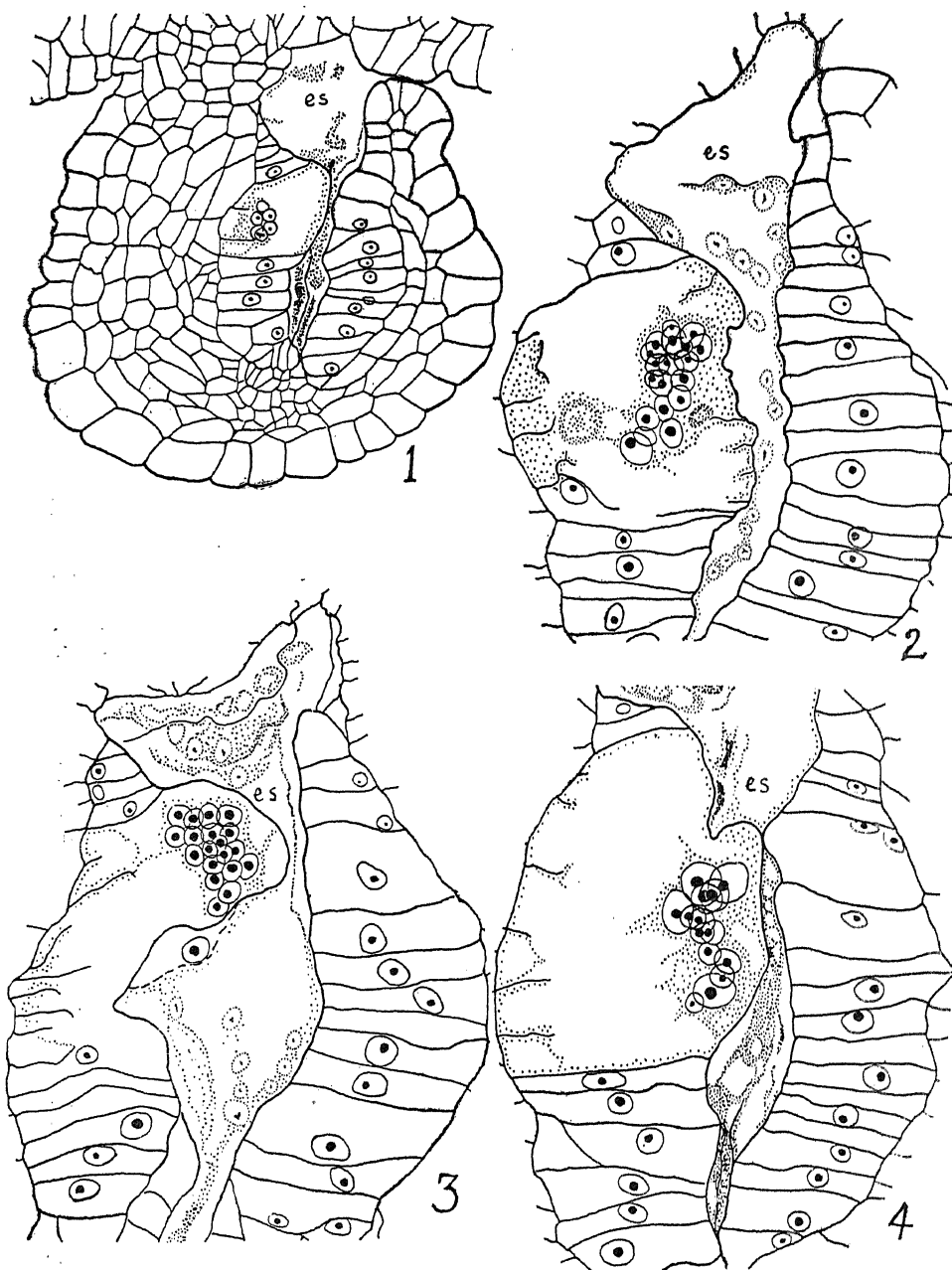
The ovules of *Utricularia flexuosa* Vahl are anatropous, unitegmic and tenuinucellate. They are borne on a spherical placenta which shows a group of nutritive cells near the base of each ovule. With the development of the embryo sac the nucellar cells are absorbed so that it comes in contact with the inner epidermis of the integument. The latter develops into a conspicuous endothelium. The tip of the gametophyte grows beyond the integument and invades the placenta, coming in close contact with the nutritive tissue (Khan, 1954). It has been reported earlier that the phenomenon of degeneration occurs frequently in *Utricularia flexuosa* and that it may affect the ovule or the various structures that develop within it, namely, the female gametophyte, the endosperm and the embryo (Khan, 1963). The ovules which contain the degenerating female gametophytes often exhibit an unusual behaviour of cells belonging to the integumentary tapetum. An account of this interesting phenomenon is presented below.

**Material and Methods :**

The material of *Utricularia flexuosa* was collected from the Najafgarh drain in the suburbs of Delhi. Formalin-acetic-alcohol and Nawashin's fluid were used as fixatives. The usual methods of dehydration, infiltration and embedding in paraffin were employed. Sections were cut at a thickness of about  $10\mu$  and stained with safranin and fast green as well as in iron alum haematoxylin.

**Observations :**

In many ovaries, numerous ovules developed into seeds but in other ovules the embryo sac remained unfertilized. The nuclei of some of the unfertilized embryo sacs showed various degrees of degeneration. In older stages, nothing but a degenerated mass was seen in the embryo sac or even this mass disappeared so that the only trace of an embryo sac left was a small cavity with cytoplasm including a few starch grains. The healthy embryo sac is broad in the micropylar region, tapering towards the chalaza. The integumentary tapetum surrounds the embryo sac almost completely with the exception of its two extremities. The cells of the integumentary tapetum situated around the micropylar part of the embryo sac are small while those situated in the middle and the chalazal region are large and constitute a well-defined region of the tapetum. It is the cells in the beginning of the well-defined region on the funicular side of the embryo sac that exhibited the unusual behaviour in the ovules in which the embryo sac had degenerated. The transverse walls between the cells, i.e., the walls situated at right angles to the axis of the embryo sac, broke down and the nuclei of these cells came to lie together in a common cavity (Fig. 1). In earlier stages the number of cells taking



Figs. 1-4. *Utricularia flexuosa*. Fig. 1. L s. of ovule showing degenerated embryo sac (es). The funicle is on the left. Note the behaviour of some of the tapetal cells on the funicular side. Fig. 2-4. L s. of part of ovule with degenerated embryo sac and the tapetum at higher magnification, showing later stages in the unusual tapetal behaviour. Note the nodule-like tapetal protrusion into the cavity of the degenerated embryo sac in Fig. 3. (The broken line indicates the wall of the nodule as seen in a different focus). In Fig. 4, some of the nuclei in the tapetal cavity produced by breaking up of cell wall are larger than the nuclei in the unaffected cells. Fig. 1. X393. Figs. 2-4. X840.

part in this activity was small but their number increased in later stages when the lower tapetal cells also behaved similarly. Consequently the size of the cavity grew and the number of nuclei seen together increased (Fig. 2). The longitudinal wall separating this cavity from the embryo sac bulged into the cavity of the embryo sac. The bulge was sometimes so prominent and well defined as to look more or less like a nodule (Fig. 3). The number of nuclei seen together in the nodular cavity was as high as more than thirty. The nuclei were not scattered all over the space. Most of them were seen close together in a group and were usually located in the nodule-like protrusion or bulge (Fig. 3). It is possible that some of these nuclei might have been produced by division of the original nuclei but actual nuclear division was not observed and therefore a definite conclusion cannot be drawn.

The phenomenon has been observed in more than thirty ovules in three ovaries. Two thirds of the ovules showing this interesting phenomenon occurred in one ovary which also contained ovules exhibiting very advanced stages in degeneration. It is noteworthy that the ovules which showed the unusual tapetal behaviour were quite healthy and normal except that the female gametophyte had degenerated. The breaking down of the walls of the tapetal cells is not a degeneration phenomenon but the sign of a new activity. The form of the nodule in some cases suggested not merely a stretching of the wall but actual growth (Fig. 3). The nuclei were perfectly healthy and normal like the nuclei of those tapetal cells that had not broken down. The nuclei were as large as those in the unaffected tapetal cells and sometimes were even larger (Fig. 4.)

A distinct feature of the phenomenon is its occurrence in a definite region of the ovule on the funicular side. In a very few cases, it was not possible to decide whether the phenomenon occurred on the funicular or the non-funicular side. Out of more than thirty cases observed, it was only in one or two ovules that the phenomenon was seen on the side away from funicle. The unusual tapetal behaviour is ordinarily seen in ovules with degenerating gametophytes but a later developmental stage was noted in one case. It was an old ovule and contained endosperm and embryo, the endosperm being in a degenerated condition although the embryo embedded in it was healthy.

#### **Discussion :**

It is difficult to explain either the cause or the significance of the unusual and interesting behaviour of the tapetum described above. So far this phenomenon has not been observed in ovules containing healthy structures. The largest number of cases was seen in one ovary containing many degenerating ovules. The phenomenon thus would seem to be the result of degeneration inside the ovule alone or it may be further stimulated by degeneration in other ovules in the ovary. It is also possible that the peculiar tapetal behaviour and the degeneration of various structures may be the result of a common cause.

The peculiar tapetal behaviour is not an artefact. Sometimes, during the preparation of the slides, the microtomed sections may become variously distorted due to defective stretching of the paraffin ribbons but, in the present case, this does not explain the peculiar tapetal behaviour. The phenomenon was seen in those ovules only that contained degenerating structures. It occurred almost always on the same side of the ovule and more or less in the same region. Moreover, when the unusual tapetal condition in an ovule extended to two or more consecutive sections, it was exactly the same spot, in the same ovule, that was seen to be affected in the consecutive sections while the intervening regions

were unaffected. Defective stretching cannot be expected to be so selective in producing the artefact. The nuclei tended to converge to one place and were not scattered apart as would be expected if it was a mere distortion produced by overheating of the paraffin ribbons.

The degeneration of the embryo sac might possibly cause the tapetum to expand and bulge inwards by releasing the pressure, if any, that it might have been exerting upon the tapetum when it was in a healthy state. But if this were really so, the entire tapetum would have exhibited an inward expansion from all directions and not in a particular spot only. Is there any significance in the fact that the phenomenon occurs almost always on the funicular side? The role of the funicle in the food supply of the ovule is not very significant in this plant because the funicle is devoid of the vascular supply. The embryo sac gets its nourishment mainly from the placenta directly from the placental nutritive tissue situated at the base of each ovule.

The author is not aware of the occurrence of a similar phenomenon in other angiosperms although some comparable instances may be cited. In the Podostemaceae, disintegration of nucellar cells produces a cavity with several free nuclei, described as pseudo embryo sac, which is situated below the true embryo sac (Hammond, 1937; Razi, 1949, 1955; Mukkada, 1962). Adventive embryos developing from the cells of the integument (see Maheshwari, 1950) or the integumentary tapetum (see Johansen, 1950) have been reported but, according to some, they are only tumorous growths. Naphthaleneacetic acid or indolebutyric acid injected into the ovaries of *Datura stramonium* induces parthenocarpic fruits with greatly enlarged ovules which develop seed coats and often contain a "pseudoembryo" consisting of several hundred cells. The "pseudoembryo" originates by proliferation from the inner layer of the integument (integumentary tapetum) surrounding the embryo sac (Van Overbeek, Conklin and Blakeslee, 1941). Is it possible that, in *Utricularia flexuosa*, the tapetum receives, from some source, a substance or substances that stimulate it to an unusual activity? The nodule-like protrusion developing from the endothelium in *U. flexuosa* reported here would have been comparable to the "pseudoembryo" described by Van Overbeek, Conklin and Blakeslee (1941), had it been a cellular structure and not free nuclear.

#### Summary :

An unusual behaviour of the cells of the integumentary tapetum, situated on the funicular side of the embryo sac near its upper or middle region, has been observed in a large number of ovules containing degenerating gametophytes. The transverse walls of these cells break down and their nuclei come together in a common cavity. The longitudinal wall separating the cavity from the embryo sac bulges into the embryo sac more or less like a nodule and the nuclei are located in the nodule-like protrusion. The nuclei are quite healthy and normal in appearance and may even show an increase in size. The number of nuclei seen together in a nodule may exceed thirty.

The phenomenon is not an artefact or a degeneration process. It indicates a new activity whose cause or significance needs an explanation.

### Literature Cited :

- Hammond, B. L. Development of *Podostemon ceratophyllum*. *Bul. Torrey Bot. Club*, **64** : 17-36, 1937.
- Johansen, D. A. Plant Embryology. Embryogeny of the Spermatophyta. Waltham, Mass. (U. S.A.), 1950.
- Khan, Reayat. A contribution to the embryology of *Utricularia flexuosa* Vahl. *Phytomorphology*, **4** : 80-117, 1954.
- The phenomenon of degeneration in the ovules of *Utricularia flexuosa* Vahl. *Proc. Indian Sci. Cong. Golden Jubilee Session*, Part III Abstracts : 382-383, 1963.
- Maheshwari, P. An Introduction to the Embryology of Angiosperms. New York, 1950.
- Mukkada, A. J. Some observations on the embryology of *Dicraea stylosa* Wight. *Plant Embryology*. A symposium. C. S. I. R., New Delhi, 1962.
- Razi, B. A. Embryological studies of two members of the Indian Podostemaceae. *Bot. Gaz.*, **111** : 211-218, 1949.
- Some aspects of the embryology of *Zeylanidium olivaceum* (Tul.) Engl. and *Lawia zeylanica* Tul. *Bull. Bot. Soc. Beng.*, **9** : 36-41, 1955.
- Van Overbeek, J., Conklin, M. E. and Blakeslee, A. F. Chemical stimulation of ovule development and its possible relation to parthenogenesis. *Amer. J. Bot.*, **28** : 647-656, 1941.



### **EDITORIAL BOARD**

1. Prof. P. Maheshwari, New Delhi (*Chairman*)
2. Padma Bhushan Dr. B. N. Prasad, Allahabad
3. Dr. P. L. Srivastava, Muzaffarpur
4. Prof. A. K. Bhattacharya, Sagar
5. Prof. N. R. Dhar, Allahabad
6. Prof. S. Ghosh, Jabalpur
7. Prof. R. N. Tandon, Allahabad
8. Prof. M. D. L. Srivastava, Allahabad
9. Prof. S. M. Das, Srinagar
10. Prof. Raj Nath, Varanasi
11. Prof. S. N. Ghosh, Allahabad
12. Dr. R. K. Saxena, Allahabad (*Secretary*)

## CONTENTS

Nutritional requirements of <i>Alternaria tenuis</i> Auct. causing leaf spot disease of Tomato	R. N. Tandon and C. Chaturvedi	4
The physiology of mixed cropping	Amar Singh	4
Effect of nitrogen starvation on multiplication of Active Tobacco Mosaic virus in Turkish Tobacco ( <i>Nicotiana tabacum</i> )	J. P. Varma and G. S. Verma	4
Studies in the Taxonomy and Cytology of certain species of <i>Aneilema sensu lato</i> in Eastern India	G. Panigrahi and R. V. Kammathy	4
Biometrical grouping in <i>Protomyces macrosporus</i> Ung. causing stem-gall disease of Coriander	J. S. Gupta and S. Sinha	50
The growth and yield of <i>Triticum aestivum</i> under the influence of Gibberellic acid.	Amar Singh	51
Control of loose smut of Barley—By seed treatment	R. S. Mathur	52
The origin and utility of some vernacular plant names.	S. K. Jain	525
On the Structure and Mechanism of the Genitalia of <i>Aulacophora</i> .	R. S. Saini	531
Effect of some physical factors on the genus <i>Onychiurus</i> (Collembola)	D. K. Choudhuri	539
The utilization of raffinose by some pathogenic species of <i>Phyllosticta</i>	K. S. Bilgrami	547
Influence of different soil temperatures on the incidence of <i>Fusarium</i> Wilt of Gram ( <i>Cicer arietinum</i> L.)	S. K. Chauhan	552
Morphology of the Thorax of Araeopidae (Homoptera, Fulgoroidea)	R. D. Saksena and A. N. T. Joseph	555
Effect of Caffeine on flowering of <i>Calendula Officinalis</i> —A Note.	D. M. Verma	570
Role of manganese in the growth of root and shoot of <i>Gajanus cajan</i>	Amar Singh and Archana Pal	571
Some physiological studies on <i>Pestalotiopsis versicolor</i> (Speg.) Stey	R. N. Tandon and S. K. Mitra	583
Male reproductive organs of <i>Lathrecista asiatica asiatica</i> Fabricius (Libellulidae : Odonata)	Brijesh Kumar Srivastava	591
A new Indian species of <i>Peripsocus</i> Hagen, 1866 (Psocoptera : Peripsocidae).	B. Datta	606
Fishes of Chotanagpur (Part I), Sadar Sub-Division Ranchi, Bihar	C. M. Srivastava and V. R. P. Sinha	611
Effect of certain fungicides, Antibiotics and Synthetic phytohormones on the germination of chlamydospores of <i>Protomyces macrosporus</i> Unger causing 'Stem-gall' disease of Coriander	S. B. Mathur and P. N. Narula	615
Smut disease of 'Sawan', <i>Echinochloa frumentacea</i> Link caused by <i>Ustilago paradoxa</i> Syd. and Butl.	B. B. Sharma	616
The digestive system of some British Amphipods Part II (Description of mouth parts)	V. P. Agrawal	63
The localization of vernalization influences	S. C. Chakravarti	64
The heart of Indian Cuchia Eel, <i>Amphipnous cuchia</i> (Ham)	H. S. Chaudhry and A. B. Das	64
The behaviour of the Integumentary tapetum in the Ovules containing degenerating gametophytes in <i>Utricularia flexuosa</i> Vahl	Reayat Kuan	

Published by Dr. R. K. Saksena, for the National Academy of Sciences, India, Allahabad  
and Printed by E. K. Raja at The Capital Printing Works, Allahabad.  
Secretary Editorial Board—Dr. R. K. Saksena.